Fractionation of agro-waste to produce biopolymers and bioactive compounds for active food packaging

by

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ABSTRACT

The food packaging industry is faced with a need to find alternative raw materials to replace the non-renewable petroleum-based polymers and active compounds used in developing active food packaging material (packaging film capable of maintaining food quality by releasing antioxidants into food in response to temperature and time) and temperature-time indicators. Agricultural residues are a promising one-stop feedstock for biopolymers (hemicellulose, nanocellulose, pectin) and active compounds (polyphenols, anthocyanins) that can be used to develop renewable, biodegradable and non-toxic active food packaging and temperature-time indicators. However, the sequential or co-extraction of these raw materials from agricultural residues is hindered by the differences in the optimal extraction conditions of the products. Furthermore, the coexistence of these products with other plant material in biomass affects their effective recovery, downstream processing and end-application. On the application side, biocomposite films such as hemicellulose-based films made from agro-derivatives, completely disintegrate when in contact with aqueous solutions, hence, they cannot be used for packaging wet food, limiting their application only to dry foods packaging. In addition, the poor mechanical properties (tensile strength and Young's modulus) of these films hinder their application as alternative packaging to petroleum-based films such as low density polyethylene (LDPE). Manipulating and optimising the biopolymers and antioxidants extraction processes taking into consideration downstream modification processes and end-application, can be a strategy in tailor-making the properties of the agro-derivatives with the aim of developing active food packaging films with properties similar or better than LDPE-based active packaging. Furthermore, modifying and blending the biopolymers to form films and applying hydrophobic coatings to the films can further enhance film properties. Therefore, the aim of the study was to develop processes for fractionating wheat straw and mango peels to recover biopolymers and bioactive compounds and then integrate the products into bio-composite films for active food packaging applications, using LDPE films as benchmark. In addition, the application of bioactive compounds from agro-residues as temperature-time indicators was evaluated.

To fulfill the aim, the first part of the project focused on optimising organosolv pre-treatment of wheat straw [ethanol (50–80 %)/sodium hydroxide (NaOH) (1–13%)/2–6 h] prior to hemicellulose extraction (10% NaOH/25 °C/24 h) so as to enhance hemicellulose acetylation, hydrophobicity, and the mechanical properties of hemicellulose-based films. A three-stage sequential extraction process was developed and optimised to recover anthocyanins (50–80% ethanol/0.1–2% acetic acid/25 °C/60–150 min), polyphenols (65–85% ethanol/75 °C/20–60 min) and pectin (0.25% ammonium oxalate-oxalic acid/85 °C/60 min) from mango peels. The second part involved the development and evaluation of hemicellulose-based films as active packaging material. The utilisation of a

combination of nanocellulose (25% w/w) and pectin (hemicellulose/pectin ratio from 0% to 100%) as reinforcement and filler in hemicellulose-based films was investigated. Furthermore, the effects of modifying hemicellulose by acetylation, reinforcing the acetylated hemicellulose (AH) with acetylated nanocellulose (ACNC) of varying degrees of acetylation (DS 0–2.34) and loading (10–50%) and coating the AH/ACNC films with polycaprolactone (PCL) (0.3 g/L) on the films' mechanical properties, hydrophobicity and solubility in wet food simulants were assessed. Hemicellulose-based active packaging material was then formulated by doping the films with mango peel polyphenols. Antioxidant release by the hemicellulose-based active packaging material to different food simulants, was evaluated both experimentally (temperature range 5–40 ℃ for two days) and by using Migratest Exp. LDPE films doped with polyphenols were used as a benchmark. Lastly, the utilisation of anthocyanins as a time-temperature indicator was investigated by evaluating colour change of the anthocyanin/hydrogen phosphate buffer solution incubated at temperatures ranging from 5 ℃ to 40 ℃ for three days.

Organosolv pre-treatment optimal conditions [(1% NaOH/50% ethanol/6 h/75 ℃)] of wheat straw enhanced the acetylation of the recovered hemicellulose (DS increased from 1.2 to 1.7) and in turn improved water resistivity (water contact angle (WCA) increased from 34.21° to 39.90°) of hemicellulose-based films. Increasing the reinforcing nanocellulose DS to 2.34, loading to 50% and coating the AH/ACNC films with PCL increased the films' Young's modulus from 55.23 to 335.33 MPa, tensile strength from 1.79 to 6.72 MPa, and WCA from 39.90° to 82.40°. There was no significant difference ($p > 0.05$) between the aforementioned results and the LDPE properties (Young's modulus 244.46 MPa, tensile strength 11.07 MPa and WCA 84.91°). The solubility of the hemicellulose-based films in the food simulants was reduced from 100% to 3.25% by the aforementioned modifications. Blending hemicellulose with pectin and nanocellulose improved the mechanical properties and reduced the solubility of the films in the fatty food simulant by up to 84%. Hemicellulose-based films released up to 98.54% whereas LDPE released 6.89% of the encapsulated polyphenols into food simulants, thereby increasing the antioxidant activity of the food simulant by up to 60% and 8.97% respectively. AH-based films were least soluble and had the highest antioxidant release in the fatty simulant when compared to the other simulants. Therefore, hemicellulose-based films can be utilised as alternative active packaging for short term storage (0–2 days) of fatty foods. Mango anthocyanins changed colour from orange to dark green with an increase in temperature from 0 ℃ to 40 ℃ and time 0 to 2 days, hence exhibiting temperature-time indicator properties.

Overall, the study generated novel methods for manipulation of hemicellulose to increase its applications as a functional food packaging material. New information was generated when

hemicellulose films were tested as active packaging in a food environment. This work also contributed to the development of agro-residue biorefineries for multiple product production and their utilisation for sustainable food packaging material development.

OPSOMMING

Die voedselverpakkingsindustrie staar ’n noodsaaklikheid in die gesig om alternatiewe roumateriale te vind om nie-hernubare petroleum-gebaseerde polimere en aktiewe samestellings wat in die ontwikkeling van aktiewe voedselverpakkingsmateriaal (verpakkingsfilm in staat om voedselkwaliteit te handhaaf deur anti-oksidante binne-in voedsel vry te laat in respons tot temperatuur en tyd) en temperatuur-tyd indikators gebruik word, te vervang. Landbou-residu’s is ’n belowende een-stop voermateriaal vir biopolimere (hemisellulose, nanosellulose, pektien) en aktiewe samestellings (polifenole, antosianien) wat gebruik kan word om hernubare, bioafbreekbare en nie-toksiese aktiewe voedselverpakking en temperatuur-tyd indikators te onwikkel. Die sekwensiële of koëkstrahering van hierdie rou materiale vanuit landbou-residu’s word egter verhinder deur die verskille in die optimale ekstraheringskondisies van die produkte. Verder, die kobestaan van hierdie produkte met ander plantmateriaal in biomassa affekteer hul doeltreffende ontginning, stroomaf prosessering en eindelike toepassing. Aan die toepassing se kant disintegreer biosaamgestelde films heeltemal, soos hemisellulose-gebaseerde films gemaak uit landbou derivate, wanneer dit in kontak kom met waterige oplossings, en dus kan dit nie vir verpakking van nat voedsel gebruik word nie, wat die toepassing daarvan slegs tot droë voedsel beperk. Boonop verhinder die swak meganiese eienskappe (treksterkte en Young se modulus) van hierdie films hul toepassing as alternatiewe verpakking teenoor petroleum-gebaseerde films soos ’n lae digtheid poliëtileen (LDPE). Deur die biopolimere en anti-oksidante se ekstraksieproses te manipuleer en optimeer, met die inagneming van stroomaf modifikasieprosesse en eindelike toepassing, kan ’n strategie wees om die eienskappe van die landbou derivate spesifiek te maak met die doel om aktiewe voedselverpakkingsfilms met eienskappe soortgelyk of beter as LDPE-gebaseerde aktiewe verpakking te ontwikkel. Verder, deur die biopolimere te wysig en te meng om films te vorm en hidrofobiese bedekking op die films toe te pas, kan die eienskappe van die films verder verbeter. Daarom was die doel van hierdie studie om prosesse te ontwikkel vir fraksionering van koringstrooi en mangoskille om biopolimere en bioaktiewe samestellings te herwin en dan die produkte by die biosaamgestelde films te integreer vir aktiewe voedselverpakkingtoepassings, deur LDPE-films as maatstaf te gebruik. Boonop is die toepassing van bioaktiewe samestellings van landbou-residu’s as temperatuur-tyd indikators geëvalueer.

Om die doel te bereik het die eerste deel van die projek op die optimering van organsolv voor-behandeling van koringstrooi (etanol (50–80%)/seepsoda (NaOH) (1–13%)/2–6 h) voor hemisellulose-ekstrahering (10% NaOH/25 °C/ 24 h) om die hemisellulose se asetilasie, hidrofobisiteit en die meganiese eienskappe van hemisellulose-gebaseerde films te verbeter. ’n Drie-fase sekwensiële ekstraksieproses is ontwikkel en geoptimeer om antosianiene (50–80%

etanol/0.1–2% asynsuur/25 °C/60–150 min), polifenole (65–85% etanol/75 °C/20–60 min) en pektien (0.25% ammonium oksalaat-oksaalsuur/85 °C/60 min) van mangoskille te herwin. Die tweede deel het die ontwikkeling en evaluasie van hemisellulose-gebaseerde ingesluit films as aktiewe verpakkingsmateriaal. Die gebruik van ’n kombinasie van nanosellulose (25% w/w) en pektien (hemisellulose/pektien ratio van 0% tot 100%) as versterking en vuller in hemisellulose-gebaseerde films is ondersoek. Verder, is die effek om hemisellulose by asetilasie te wysig, versterking van geasetileerde hemiselluslose (AH) met geasetileerde nanosellulose (ANCN) van variërende grade van asetilering (DS 0–2.34) en lading (10–50%) en bedekking van die AH/ACNC-films met polikaprolaktoon (PCL (0.3 g/L) op die film se meganiese eienskappe, hidrofobisiteit en oplosbaarheid in nat voedselsimulante geassesseer. Hemisellulose-gebaseerde aktiewe verpakkingsmateriaal is toe geformuleer deur die films met mangoskilpolifenole te dokter. Anti-oksidant vrylating deur die hemisellulose-gebaseerde aktiewe verpakkingsmateriaal na verskillende simulante is geëvalueer, beide eksperimenteel (temperatuurbestek 5–40 °C vir twee dae) en deur Migratest Exp. te gebruik. LDPE-films gedokter met polifenole is gebruik as maatstaf. Laastens is die gebruik van antosianiene as ’n tyd-temperatuur indikator ondersoek deur kleurverandering van die antosianien/waterstoffosfaat-bufferoplossing geïnkubeer by temperature in die bestek van 5 °C tot 40 °C vir drie dae, te evalueer.

Organosolv voor-behandeling (optimale kondisies (1 NaOH/50% etanol/6 h/75 °C)) van koringstrooi het die asetilering van die herwinde hemisellulose verbeter (DS verhoog van 1.2 na 1.7) en op sy beurt waterresistiwiteit (waterkontakhoek (WCA) verhoog van 34.21° na 39.90°) van hemisellulose-gebaseerde films verbeter. Deur die nanosellulose DS na 2.34 te verhoog, te laai na 50% en die AH/ACNC-films met PCL te versterk, het die films se Young se modulus van 55.23 na 335.33 MPa verhoog, treksterkte van 1.79 na 6.72 MPa, en WCA van 39.90° na 82.4°. Daar was geen beduidende verskil ($p > 0.05$) tussen die voorafgenoemde resultate en die LDPE-eienskappe nie (Young se modulus 244.46 MPa, treksterkte 11.07 MPa en WCA 84.91°). Die oplosbaarheid van die hemisellulose-gebaseerde films in die voedselsimulante is verlaag van 100% na 3.25% deur die voorafgenoemde wysigings. Deur hemisellulose met pektien en nanosellulose te meng, het die meganiese eienskappe verbeter en die oplosbaarheid van die films in die vetterige voedselsimulant tot en met 84% verminder. Hemisellulose-gebaseerde films het tot en met 98.54% vrygelaat waar LDPE 6.89% van die vasgevangde polifenole in die voedselsimulante vrygelaat het, en daardeur die anti-oksidantaktiwiteit van die voedselsimulant tot en met 60% en 8.97%, onderskeidelik, verhoog het. AH-gebaseerde films was die minste oplosbaar en het die hoogste anti-oksidant vrystelling gehad in die vetterige simulante wanneer dit met ander simulante vergelyk geword het. Daarom kan hemisellulose-gebaseerde films gebruik word as alternatief vir korttermynbewaring (0–2 dae) van

vetterige voedsel. Mango-antosianiene het kleur van oranje na donker groen verander met ’n verhoging in temperatuur van 0 °C tot 40 °C en tyd 0 tot 2 dae, en daarom temperatuur-tyd indikator eienskappe vertoon.

Algeheel het die studie nuwe metodes vir manipulering van hemisellulose gegenereer om sy toepassings as ’n voedselverpakkingsmateriaal te verhoog. Nuwe informasie is gegenereer toe hemisellulose films getoets is as aktiewe verpakking in voedselomgewings. Hierdie werk het ook tot die ontwikkeling van landbouresidubioraffinaderye vir veelvoudige produk vervaardiging en hul gebruik vir volhoubare voedselverpakkingsmateriaal bygedra.

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DEDICATION

I dedicate this thesis to my husband Lameck and my children Nathaniel and Mutsawashe.

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Abbreviations and Symbols

1. LDPE	Low density polyethylene
2. PCL	Polycaprolactone
3. AH	Acetylated hemicellulose
4. ACNC	Acetylated nanocellulose
5. CNC	Nanocellulose
6. DMSO	Dimethyl sulfoxide
7. AGX	Arabino 4-O-glucurunoxylan
8. OPT	Optimum conditions
9. DS	Acetylation degree of substitution
10. UAC	Uronic acid content
11. WCA	Water contact angle
12. DE	Degree of esterification
13. FTIR	Fourier transform infrared spectroscopy

1. INTRODUCTION

There has been a positive drive towards finding alternative raw materials for the production of food packaging films to reduce the reliance on non-renewable fossil resources (Arrieta *et al.*, 2014; Huang *et al.*, 2018). Fossil-based plastic films such as LDPE are commonly used as shrink wraps, bags, and pouches in the food packaging industry because of their flexibility (modulus of elasticity ranging between 0.172–0.282 GPa), percent elongation (100–650), tensile strength (8.3–31.4 MPa) and hydrophobicity (WCA ranging between 90^{o}–128^{o}) (Plackett, 2011; Shit and Shah, 2014). However, the food packaging industry is moving towards the utilisation of biobased films as a substitute for LDPE films. This is due to the research findings that toxic monomers such as ethylene and additives such as bisphenol in LDPE films can migrate into the packaged food, thus compromising the health of consumers (Sanches Silva *et al.*, 2007; de Abreu *et al.*, 2009). In addition, LDPE-based films are a threat to the environment if not properly disposed of because they can remain unchanged in the environment for 10–32 years. Currently, LDPE films are disposed of by incineration to recover energy, however toxic chemicals such as dioxins, which are potentially carcinogenic, are released into the environment (Mandal, 2005; Krzysztof Pikoń, 2014). In a bid to protect consumers, the environment and reduce reliance on LDPE films (0.2% biodegradation in 10 years) in food packaging, hemicellulose-based films (non-toxic plant derivative which are 100% biodegradable in 200 days) (Muniyasamy *et al.*, 2013; Nguyen *et al.*, 2016), were developed and tested in this study as potential food packaging materials.

Various studies have highlighted that hemicellulose films can be a replacement for petroleum-based films because of their barrier properties, biodegradability and abundance, although their application has not been evaluated in a food environment (Mikkonen and Tenkanen, 2012; Shimokawa *et al.*, 2015, Lasure, Min and Rosenberg, 2004; Chen, 2014). The hemicellulose types that have been used in developing food packaging films are mannans and xylans because of their ability to be moulded into films (Mikkonen and Tenkanen, 2012). Shimokawa *et al.* (2015) identified xylans, specifically water-soluble xylans, as more suitable for developing food packaging films when compared to water-insoluble xylans and mannans. This is because the water soluble xylans, such as arabino-4-O-methylglucuronoxylan (AGX), produce transparent films which are more appealing to customers than opaque films developed from mannans and water-insoluble xylans (Shimokawa *et al.*, 2015). Based on this fact, AGX hemicellulose recovered from wheat straw was utilised in developing food packaging material.

The commercial use of hemicellulose-based films is still limited by the poor mechanical (tensile strength ranging from 3.3 MPa to 14 MPa and Young's modulus ranging from 3 MPa to 100 MPa) (Gordobil *et al.*, 2014; Liu *et al.*, 2016) and barrier properties (WCA < 70^{o}) of the films attributed to their hydrophilic nature (Egüés *et al.*, 2014; Shimokawa *et al.*, 2015). Moisture can accumulate in the xylan structure when the films are exposed to high humidity, due to hydrogen bonding of hydroxyl groups in the AGX structure and water molecules (Akkus *et al.*, 2018). Repeated accumulation and removal of moisture from the films in response to environmental changes compromises the mechanical and barrier properties of the films (Azeredo *et al.*, 2015). Therefore, there is a need to modify hemicellulose in order to reduce the hydroxyl groups available for bonding with water. Processes such as acetylation, reinforcing the films (with fillers such as ACNC and nanoclays) and coating the films with hydrophobic material (PCL), have the potential to reduce the hydrophilicity of hemicellulose-based films (Chen *et al.*, 2016; Li and Pan, 2018).

The acetylation process involves replacing hydroxyl hydrogen atoms in hemicellulose with non-polar acetyl groups, thereby preventing hydrogen bonding of the hemicellulose with water in humid conditions (Egüés *et al.*, 2014; Gordobil *et al.*, 2014). Just like with any biopolymer modification process, the composition of the polymer plays a vital role in the effectiveness of the modification process (Paulos *et al.*, 2016). Hence, it is essential to manipulate the hemicellulose composition during or after extraction for optimal modification by acetylation. Current studies have only focused on manipulating hemicellulose composition after extraction using chemicals and enzymes (Stepan *et al.*, 2012; Li and Pan, 2018). However, although different hemicellulose compositions have been reported in literature for different biomass pre-treatment conditions, the manipulation of hemicellulose composition prior to hemicellulose extraction with the aim of enhancing the acetylation process is non-existent. Therefore, to demonstrate that hemicellulose modification by acetylation can be manipulated before hemicellulose extraction, the effect of organosolv pre-treatment of wheat straw prior to hemicellulose extraction on hemicellulose acetylation, hydrophobicity and compatibility with ACNC, was studied (Scarlat *et al.*, 2010; Peng, Peng, Xu and Sun, 2012).

Research on AH reinforced with ACNC is still limited to the work done by Egüés, Stepan, Eceiza and Toriz, (2014) and Gordobil *et al.*, (2014). In both studies, hemicellulose was acetylated to the highest DS and reinforced with ACNC with a low DS. Gordobil *et al.*, (2014) acetylated corncob hemicellulose to a DS of 1.8 and reinforced it with nanocellulose with a DS of 0.54. The AH/ACNC films produced had improved tensile strength (51 MPa), hydrophobicity (WCA 80^{o}) and Young's modulus (3200 MPa) when compared to unmodified hemicellulose (tensile strength - 5.8 MPa, Young's modulus - 206 MPa, WCA - 57^{o}) (Gordobil *et al.*, 2014). There is potential to further

improve the properties of the AH/ACNC films by increasing the DS of ACNC beyond 0.54, increasing ACNC loading and coating the films with hydrophobic polymers (PCL). A knowledge gap exists regarding the compatibility of AH and ACNC of DS above 0.54. Moreover, the application of AH-based films as a food packaging film is still lacking. Hence, the effects of hemicellulose acetylation (DS 0-1.7), reinforcing the AH with ACNC of different DS (0–2.34) and increasing ACNC loading from 10% to 50% on the AH-based films' hydrophobicity, mechanical properties and stability when in contact with food simulants were evaluated. Furthermore, the effect of coating the reinforced films with PCL on the aforementioned film properties, was assessed.

More importantly, there has been a shift towards enhancing food packaging material to perform beyond just being a protective barrier. The packaging material should also be able to manipulate and detect changes in the quality of packaged food, thus fulfilling the role of active packaging (Maciel, Yoshida and Franco, 2012; Prasad and Kochhar, 2014; Pereira, de Arruda and Stefani, 2015). Deviation of food quality from the optimum is facilitated by pathogen growth and oxidative reactions within the packaged food facilitated by variations in food storage temperature (Prasad and Kochhar, 2014a). Food packaging material capable of releasing antioxidants in response to temperature and time and the utilisation of temperature-time indicators can greatly increase food shelf life and promote consumer safety by reducing food spoilage agents and detecting changes in storage conditions, respectively. Plant derived and petrochemical-based active compounds have been integrated into food packaging material, with the aim of developing active food packaging to reduce food spoilage. Polyphenols and anthocyanins are gaining more popularity over their petroleum-based counterparts as additives to packaging material and temperature-indicators, respectively, because they are biodegradable, less toxic and are produced from renewable sources (Valdés *et al.*, 2014; Berardini, Fezer, *et al.*, 2005; Maciel, Yoshida and Franco, 2012).

Mango peels, particularly from red coloured cultivars, are among the promising sources of polyphenols and anthocyanins. The utilisation of mango peel extracts in developing active packaging and temperature-time indicators is still in its infancy. There is only one publication in which mango peel extracts were incorporated into PVA films, and the films evaluated as an active food packaging (Kanatt and Chawla, 2018). In addition, anthocyanins from different sources, apart from mango peels, have been incorporated into biopolymers such as chitosan and starch and evaluated as food freshness indicators (Maciel *et al.*, 2014; Prasad and Kochhar, 2014a; Pereira *et al.*, 2015). The utilisation of plant derivatives in formulating active packaging and temperature time indicators is influenced by both the source and the processes used in recovering the products. Hence, there is need to optimise the recovery of polyphenols and anthocyanins from mango peels, taking into consideration their end application as additives to food packaging and as temperature-

time indicators, respectively. Furthermore, effective extraction and utilisation of polyphenols and anthocyanins in developing active packaging material and temperature-time indicators will not only increase their functionality but also the value potential of mango peels (waste from the mango processing industry) (Banerjee *et al.*, 2017; Arora *et al.*, 2018). Therefore, this study developed a multistage extraction process for recovery of anthocyanins and polyphenols from mango peels. Residues remaining after polyphenol extraction, which are normally discarded, were utilised as a source of pectin. Hence, a novel three-stage fractionation process for mango peels was developed to recover anthocyanins, polyphenols, and pectin.

Polyphenols have been integrated into biopolymers such as cellulose, starch and chitosan and the controlled release of these antioxidants into food has been evaluated both experimentally and theoretically (López De Dicastillo *et al.*, 2011; Van Den Broek *et al.*, 2015). The purpose of testing the migration of antioxidants from food packaging into food is to check the material's compliance with laws that govern migration of chemicals from packaging material, for example the EU 10/2011. The EU 10/2011 states that the overall chemical migration limit for food packaging material is 10 mg/dm^3 on the contact area basis or 60 mg/kg of simulant (EU, 2011). These laws were gazetted to minimise health hazards associated with ingesting chemicals above the permissible concentration limit (O'Brien and Cooper, 2001; Galotto and Ulloa, 2010; EU, 2011). There is no research on the development and evaluation of hemicellulose-based films with mango peel polyphenols, in particular, AH/ACNC and hemicellulose/pectin/nanocellulose films, as active food packaging. Therefore, it is worth exploring the potential application of hemicellulose based films as active packaging material.

In order to fulfill the project's aim, the effect of wheat straw pre-treatment prior to hemicellulose extraction on hemicellulose acetylation, AH-based films' mechanical properties, hydrophobicity and compatibility with ACNC was investigated. AH films were reinforced with ACNC of different DS (0–2.34) and loading (0%–50%) and coated with PCL. The hemicellulose-based films' mechanical properties, solubility in food simulants and hydrophobicity, were then evaluated. The application of micromechanical models in predicting the mechanical properties of AH/ACNC films, was assessed. A three-stage mango peel fractionation process was developed to recover anthocyanins, polyphenols, and pectin. Polyphenols and pectin were integrated into the hemicellulose-based films and the release of antioxidants from films release into food simulants was determined both experimentally and theoretically (using migration models). The ability of mango anthocyanins to change colour in response to temperature and time was evaluated (time-temperature indicator).

2. LITERATURE REVIEW

In order to develop an active packaging material to replace LDPE-based packaging films, the properties and functionality of the LDPE films should be studied. Therefore, the current application and properties of LDPE are discussed in this section. Furthermore, when designing a new active packaging film, the methods used for recovering the raw materials (polymers and bioactive compounds) and developing the films have to be selected carefully as they play a crucial role in determining the end-application of the films (Jamshidian *et al.*, 2013). The basis for selecting hemicellulose as the packaging film matrix, its source, processes for hemicellulose recovery, modification and integration into an active food packaging are highlighted in this chapter. In addition, the methods that were applied to assess the performance of the hemicellulose-based films in a food environment are discussed.

2.1. Properties and uses of food packaging material

The major functions of packaging material are to prevent food contamination and to allow for easy handling of food (Figure 1) during transportation and storage (Han, 2005; Marsh and Bugusu, 2007; Samsudin *et al.*, 2014). In addition, packaging material has been designed to provide the consumers with information about the packaged food, that is, food composition and suggestions on food preparation and storage (Figure 1). Contamination of food can occur due to unlawful alteration, and when it is exposed to environmental conditions (temperature, pH, air) that facilitate the accumulation of food spoilage agents such as microorganisms (bacteria and fungi) and free radicals (oxygen radicals) (Marsh and Bugusu, 2007; Odeyemi *et al.*, 2020; Romani *et al.*, 2020). Micro-organisms facilitate food spoilage by boosting processes such as fermentation, whereby the sugars in food (for example fruits) are broken down into alcohol and carbon dioxide. On the other hand, free radicals react with hydrogen atoms in the lipid molecules found in food forming lipid radicals. The lipid radicals can react with oxygen to form peroxyl radicals. The peroxyl radicals can combine with a hydrogen atom from a lipid molecule to form hydroperoxide molecules. Accumulation of the hydroperoxide molecules and their breakdown derivatives such as alkoxyl and peroxyl radicals, can change the flavour of food (Desrosier and Singh, 2018).

Food spoilage consequently reduces the shelf life of packaged food. Some noticeable signs of food spoilage include discolouration, odour, and changes in texture and taste (Amit *et al.*, 2017). Khan *et al*., (2014) reported that approximately 50% of agricultural produce goes to waste annually because of packaging material which fails to prevent microbial growth, thereby resulting in food spoilage (Khan et al., 2014). Therefore, a packaging material capable of minimising the aforementioned agents of food spoilage can increase shelf life of packaged food.

Currently, most food packaging material on the market is derived from petroleum. Examples of petroleum-based packaging include LDPE, polyethylene terephthalate, high-density polyethylene, polyvinyl chloride, and polypropylene (Azeredo *et al.*, 2017). LDPE is the most widely used packaging material because of its low cost and light weight than polyethylene terephthalate, high-density polyethylene and polyvinyl chloride. Although LDPE is more expensive and heavier than polypropylene, it has the advantage of having no surface charge therefore dust and dirt cannot collect on its surface when compared to polypropylene (Goswami and Mahajan, 2009).

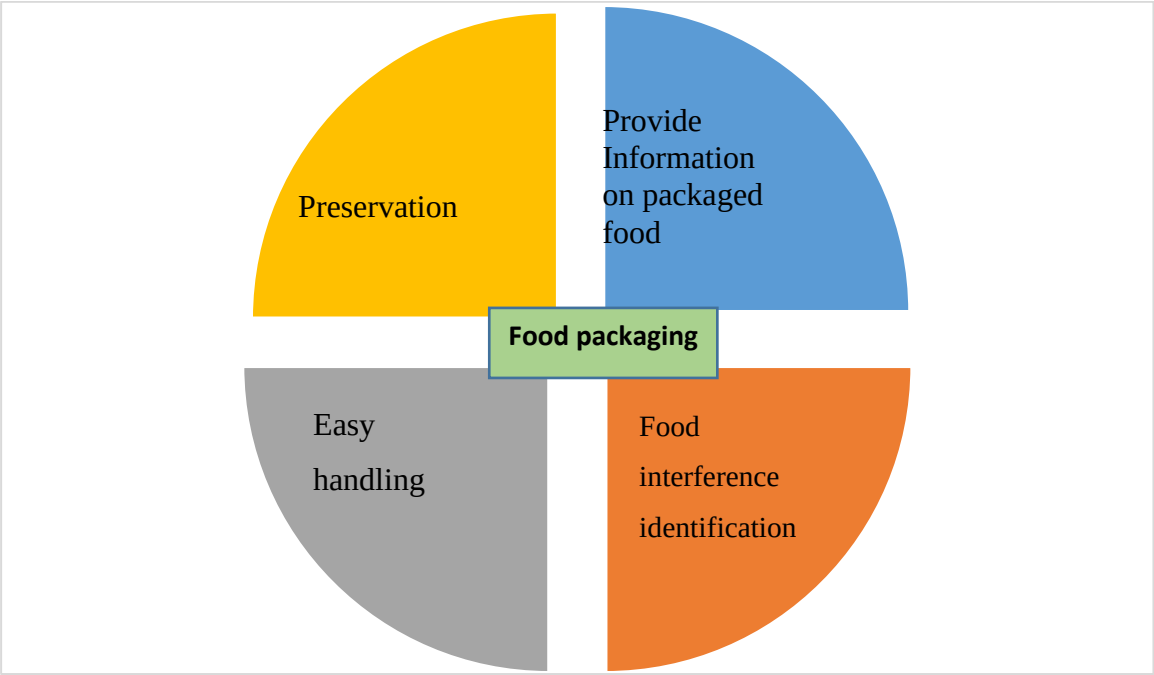

Figure 1: Application of food packaging (Kuswandi *et al.*, 2011)

2.1.1. Application of low density polyethylene as food packaging material

LDPE is a polymer chain made of ethylene units and has a density ranging from 0.91 g/cm^3 to 0.925 g/cm^3 (Jordan *et al.*, 2016). The LDPE-based films are formulated using processes such as extrusion and moulding (rotational, compression, and injection) at temperatures ranging from 115^{o}C to 400 ^{o}C (Khanam *et al.*, 2015; Yamak, 2016; Kormin *et al.*, 2017). LDPE is preferred as a food packaging material because of its light weight, resistance to chemical attack, heat sealability, excellent water barrier properties, and mechanical properties (Khanam *et al.*, 2015). The polymer has glass transition and melting temperatures of -120 ^{o}C and 105 - 115 ^{o}C respectively. Due to the LDPE films' inertness to chemical attack they have been used as packaging materials for acidic, aqueous, fatty and alcoholic foods. In addition, the hydrophobicity of LDPE films enables their utilisation as packaging material for both dry and wet foods. Packaging films developed from LDPE are capable of storing food at temperatures ranging from 0 ^{o}C to 40 ^{o}C (Goswami and Mahajan, 2009). These films are used for both long and short term storage of food.

However, the major drawbacks of LDPE films is that they do not biodegrade remaining unchanged for up to 32 years after disposal, and are made from non-renewable sources (Pikoń, 2014). Utilisation of LDPE films in packaging food with a longer shelf life (> 2 days) than for packaging perishables can be a strategy in reducing the quantity of LDPE films disposed into the environment, as the films take longer to reach the environment. Application of LDPE films for packaging perishable food poses an environmental threat as the single-use and disposal of the film in a short timeframe (< 2 days) results in accumulation of the films in the environment. In addition, if the LDPE films are not properly disposed of, they can easily be scattered due to their lightweight, causing both land and water pollution. The work by Civancik-Uslu *et al.* (2019) supports this perspective, they observed that LDPE bags which were reused 10 times had a littering potential (probability of a material to be disposed incorrectly) much higher than biobased biodegradable bags used only once (577 vs 73.5) (Civancik-Uslu *et al.*, 2019).

In order to curb the environmental issues associated with LDPE films, efforts have been made to modify LDPE with the aim of making it biodegradable. Blending LDPE films with biodegradable polymers such as starch, chitosan and polylactic acid is among the techniques that were evaluated to improve the biodegradability of LDPE films (Vasile *et al.*, 2013; Kormin *et al.*, 2017; Setiawan and Aulia, 2017). Setiawan and Aulia (2017) observed a weight loss in films blended with polylactic acid after six days, proving that adding biopolymers to LDPE films enhance the films' biodegradability (Setiawan and Aulia, 2017). In addition to increasing biodegradability, blending LDPE with biopolymers relieves pressure on non-renewable petroleum sources. However, incorporating biodegradable polymers in LDPE films resulted in a reduction in the mechanical properties of the films. For example, Kormin *et al.* (2017) reported that the tensile strength of the LDPE films reduced from 7 MPa to 2 MPa upon adding 30% starch to LDPE films. Furthermore, the hydrophobicity of LDPE films was negatively affected when biopolymers were added. Yamak, (2016) reported a decline in the hydrophobicity of LDPE films by up to 15% when starch content in the LDPE films increased to 40% (Yamak, 2016).

Another way of reducing the reliance on petroleum sources could be through modifying biopolymers to match the properties of LDPE films. Hemicellulose is one of the promising biopolymers as it is biodegradable and is extracted from renewable sources such as wheat straw and corn stover (Huang *et al.*, 2018). However, hemicellulose modification with the aim of developing films with properties similar to LDPE has not yet been accomplished.

2.2. Development of hemicellulose-based food packaging films

Hemicellulose source, modification method, and film formulation procedures are important factors to consider when designing a hemicellulose-based food packaging material. Hence, it is essential to

critically review the available literature on hemicellulose in order to select processes suitable for developing hemicellulose-based food packaging films. The following section gives an overview of the criteria used in selecting the sources of hemicellulose, extraction procedures, modification, and film development method.

2.2.1. Lignocellulosic biomass as a source of hemicellulose

Lignocellulosic biomass is becoming increasingly popular as an alternative source of biopolymers such as, cellulose, lignin, and hemicellulose as it does not compete with food sources and is normally disposed of as waste, used as animal feed or manure (Kumar 2018). Among the aforementioned lignocellulosic biopolymers, hemicellulose is gaining momentum as a renewable feedstock for chemicals and biofilms production (Akkus *et al.*, 2018; Naidu *et al.*, 2018). Hemicellulose are natural biopolymers constituting a third of plant cell wall material. The heteropolymer chains consists of xylose, uronic acids, glucose, mannose galactose, arabinose and fructose (Peng, Peng, Xu and Sun, 2012; Xue *et al.*, 2012; Saini *et al.*, 2015; Li *et al.*, 2016). Lignocellulosic biomass that is commonly used as raw material for hemicellulose includes agricultural residues, waste from municipalities and wood residues from wood value-addition (Figure 2) (Galina et al., 2018). Previous researchers have recommended wood residues as the most sustainable and economic source of hemicellulose because of their high densities (up to 800 kg/m^3) and availability throughout the year. However, it is also worth exploring agricultural residues (density of up to 380 kg/m^3) as a feedstock for biopolymers (Reyes *et al.*, 1992; Makavana *et al.*, 2018; Santos *et al.*, 2018).

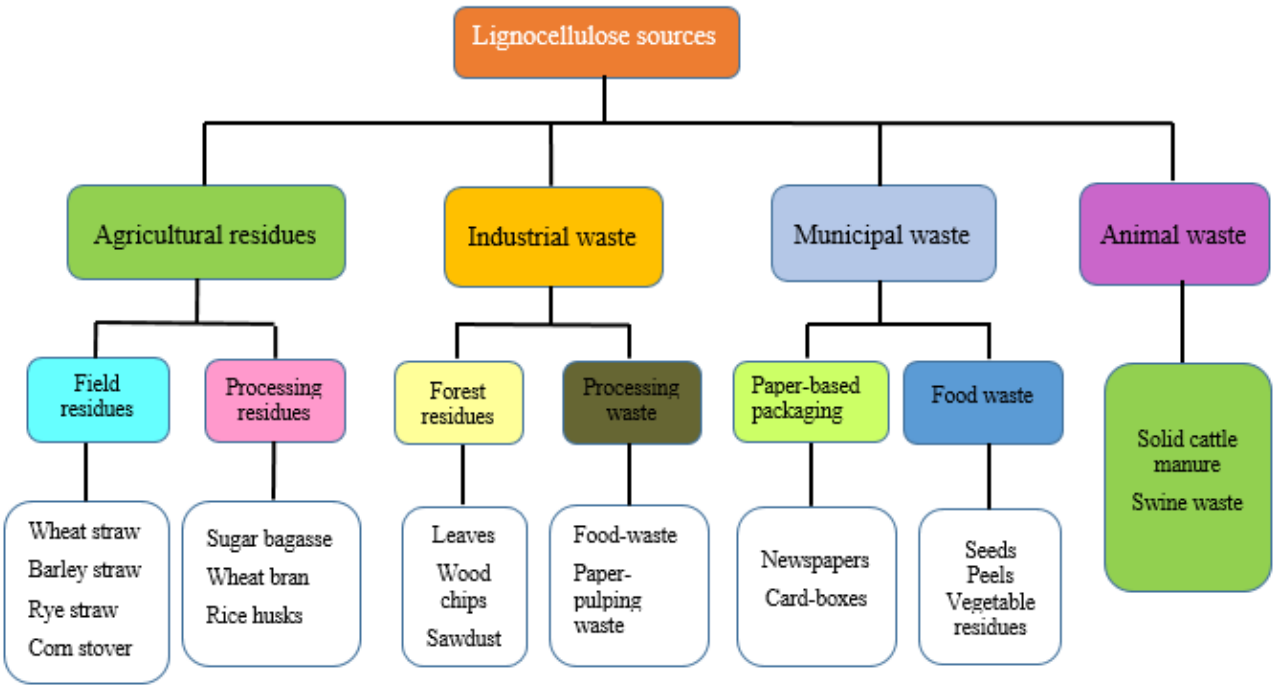

Figure 2: Types of lignocellulosic biomass

Searle and Malins, (2016) reported that agricultural residues production in Europe in 2016 was 315.9 million tonnes/year whereas forestry residues were 67.59 million tonnes/year (Searle and Malins, 2016). In Africa, approximately 40.5 million tonnes of agricultural residues and 9 million tonnes of forestry residues are produced annually (Cherubin *et al.*, 2018). South Africa alone has been reported to generate about 5.75 million tonnes/year of agricultural residues and 15.3 tonnes/year of forestry residue (Potgieter, 2011; Batidzirai, Valk, *et al.*, 2016). Evidently, agricultural residues generated in a year surpassed the wood residues produced, thus becoming a more attractive source of biopolymers. Moreover agricultural residues (Table 1) have higher hemicellulose content (25-50%) when compared to hardwoods (15-30%) and softwoods (7-12%) (Sun *et al.*, 2010a; Gomes *et al.*, 2015; Santos *et al.*, 2018). Besides the high hemicellulose content in agricultural residues, the significantly lower lignin content in these residues (5.5-20%) than wood residues (25-35%) promote the recovery of high yields of polymeric hemicellulose (Prasad *et al.*, 2007). Lignin acts as a barrier to biomass fractionation, hence, biomass with higher quantities of lignin require harsher conditions to breakdown the biomass in order to recover hemicellulose. However, in the process of removing lignin, substantial amounts of hemicellulose are degraded (Chandra *et al.*, 2015). Therefore, agricultural residues are a more attractive feedstock for recovering hemicellulose for developing hemicellulose-based food packaging films.

Agricultural residues such as barley straw, wheat straw, rice straw and corn stover have been fractionated to recover hemicellulose (Azeredo *et al.*, 2015; Hu *et al.*, 2016; Liu *et al.*, 2016; Huerta and Saldaña, 2018). Among the aforementioned agricultural residues, wheat straw is a promising feedstock for large scale recovery of hemicellulose as it has a higher hemicellulose content and it is more abundant when compared to the other residues (Table 1). According to Scarlat *et al.* (2010) the contribution of individual crop residues to the total agricultural residues produced annually in Europe were wheat straw 42.2%, while maize and barley were 18.8%, rye-3.6% and rice-1.3% (Scarlat *et al.*, 2010). Therefore, wheat straw is a potential source of hemicellulose for food packaging film production. In addition, the recovery of hemicellulose from wheat straw is a value-addition process to agricultural waste which otherwise would have had a detrimental effect on the environment if improperly disposed. For example incineration would release toxic gases such as carbon monoxide into the environment (Saini *et al.*, 2015).

Table 1: Hemicellulose content in agricultural residues

Agricultural residue	Hemicellulose content (%)	Reference
Barley straw	28.7	Hassan *et al.* (2018)
Wheat straw	38.8	Peng, Peng, Xu and Sun, (2012)
Rice straw	25.1	Wartelle and Marshall, (2006)
Corn stover	22.6	Maity, (2015)
Rye straw	36.9	Fang *et al.* (2000)
Corn cob	34.3	Ma *et al.* (2016)
Maize stalk	29.7	Menardo, Airoldi and Balsari, (2012)
Soy hull	12.5	Alemdar and Sain, (2008)

Wheat straw consists of arabino-4-O-methylglucuronoxylan (AGX) as the main hemicellulose type. AGX is a polymer chain comprising β-D-xylopyranosyl units linked together by β-(1-4) glycosidic bonds (Figure 3). This hemicellulose type contains side chains of *4-O*-methyl-α-D-glucopyranosyluronic acid, β-D-xylopyranosyl and α-L-arabinofuranosyl linked to the hemicellulose backbone by α-(1-2) glycosidic bonds. The AGX hemicellulose degree of polymerisation ranges from 100 to 200 (Pereira, 2011).

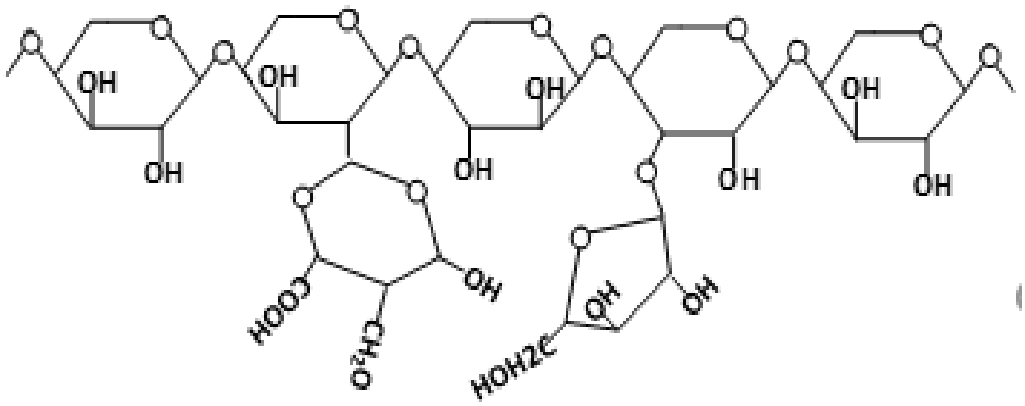

Figure 3: Structural formula of arabino-4-O-methylglucuronoxylan, drawn using Microsoft Visio (Motta, et al., 2013).

2.2.2. Hemicellulose classification and end-application

The variation of the afore-mentioned sugars in hemicellulose, attributed to the source, has led to the classification of hemicellulose as xylans, mannans, β-glucans and xyloglucans (Sun *et al.*, 2000; Belmokaddem *et al.*, 2011). For example, hardwoods can be fractionated to recover O-acetyl-(4-O-methylglucurono) xylans (Rissanen *et al.*, 2016; Stoffel *et al.*, 2017) , softwoods (for example pine

wood and spruce) can be a source of acetylated galactoglucomannans and agricultural residues (wheat straw, bagasse) (Chen *et al.*, 2015) are a feedstock for recovering glucuronoxylans (Peng, Peng, Xu and Sun, 2012; Li and Pan, 2018).

Hemicellulose composition, usually dependent on the source and extraction conditions, has been proven to influence the end-application of hemicellulose (Junli *et al.*, 2012). For example the arabinoxylans have found a use as hydrogels because of their high solubility in water resulting from the abundance of uronic acids in the hemicellulose structure (Sun *et al.*, 2010a). Xyloglucans and glucomannans have been commercially utilised as drug release agents, emulsifiers and encapsulating agents (Hansen and Plackett, 2008). Of the four classes of hemicellulose, xylans and mannans are the most suitable types of hemicellulose for formulating food packaging material based on the ability of these hemicellulose types to form flexible films. However, xylans are more widely used than mannans in developing food packaging films because xylan-based films are more transparent than mannan-based films (Shimokawa *et al.*, 2015). Film transparency and flexibility are among the major factors influencing the selection of a packaging material in the food industry (Kanatt and Chawla, 2018). Therefore, this study focused on developing xylan-based hemicellulose films for potential application as active food packaging.

2.2.3. Hemicellulose recovery from biomass

The ease of hemicellulose extraction from biomass depends on the hemicellulose source and bond strength (ionic, covalent, hydrogen and hydrophobic bonds) between the hemicellulose and other cell wall components such as lignin, cellulose, cell wall protein, and phenol compounds (Sun *et al.*, 2010; Peng, Peng, Xu and Sun, 2012). Hemicellulose to lignin covalent bonds and hemicellulose to cellulose hydrogen bonds present the greatest hindrance to hemicellulose extraction from lignocellulosic biomass. This is because of the relatively higher lignin and cellulose content in the plant cell wall when compared to the other cell wall components (Figure 4) (Sun *et al.*, 2010a).

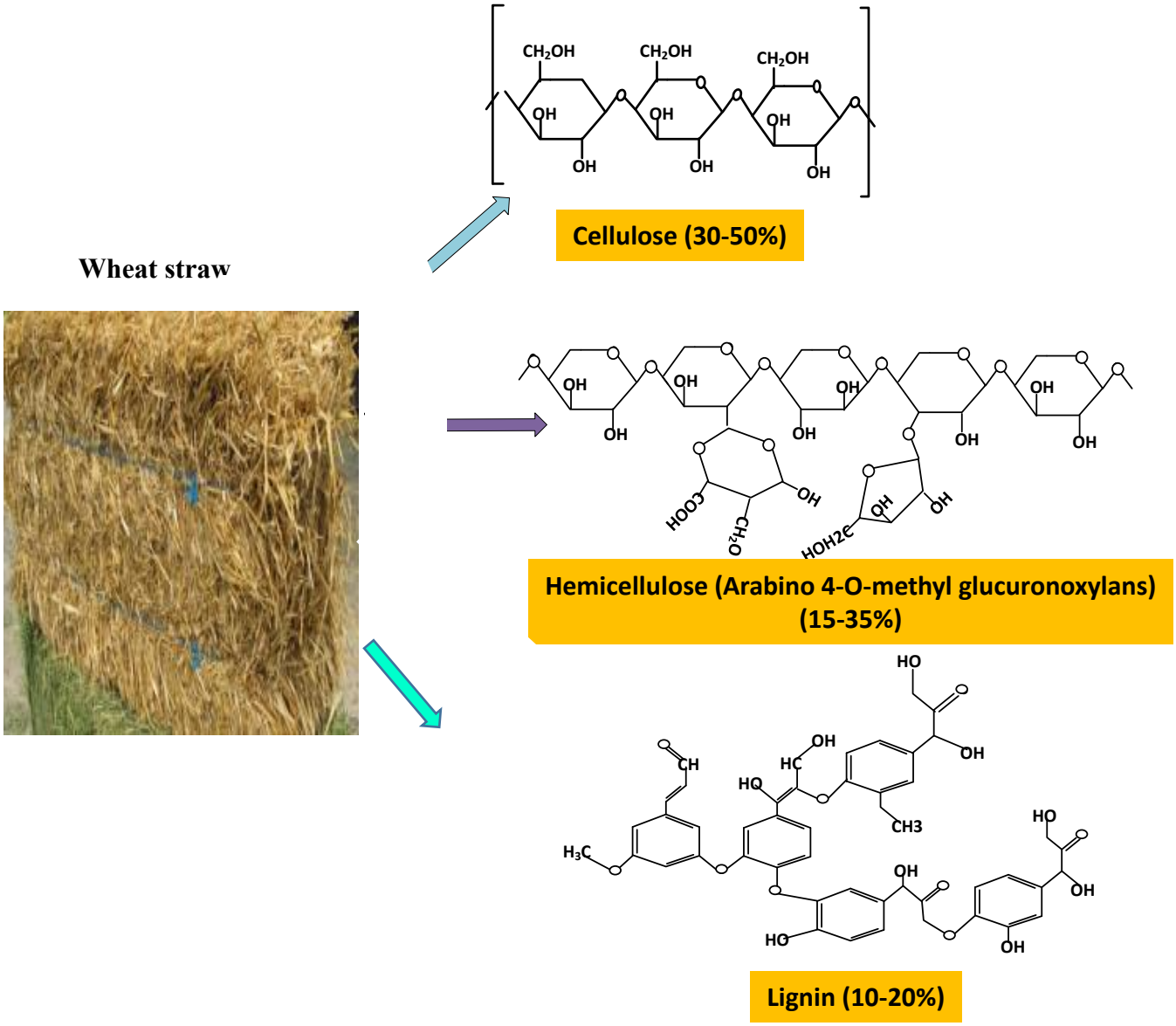

Figure 4: Composition of lignocellulosic material in wheat straw

It is easier to recover hemicellulose than the other lignocellulosic material in wheat straw, however, hemicellulose is more prone to rapid degradation during extraction. Therefore, mild extraction conditions (temperatures as low as 25 ^{o}C and alkaline conditions), have been applied to minimise the breakdown of hemicellulose to monomers, thus, ensuring that the polymeric hemicellulose required for biofilms production is recovered (Shimokawa *et al.*, 2015; Svärd *et al.*, 2015). However, harsher conditions (high temperatures, use of acids and high concentrations of alkaline solutions) are used when extracting hemicellulose for fermentation into ethanol (Brar *et al.*, 2016) and for production of value-added chemicals (Figure 5) such as hydrogels, xylitol and furfural. This is because for these applications, the monomeric form of hemicellulose is required (Ouyang, 2018). Hence, selection of a biomass fractionation method for recovery of hemicellulose depends on the end-application of the hemicellulose. There a is need to control the biomass fractionation methods in order to recover hemicellulose with specific attributes desirable for their

end-use. Therefore, hemicellulose extraction from wheat straw was manipulated and optimised for recovery of the polymeric form of hemicellulose with a composition suitable for packaging films development.

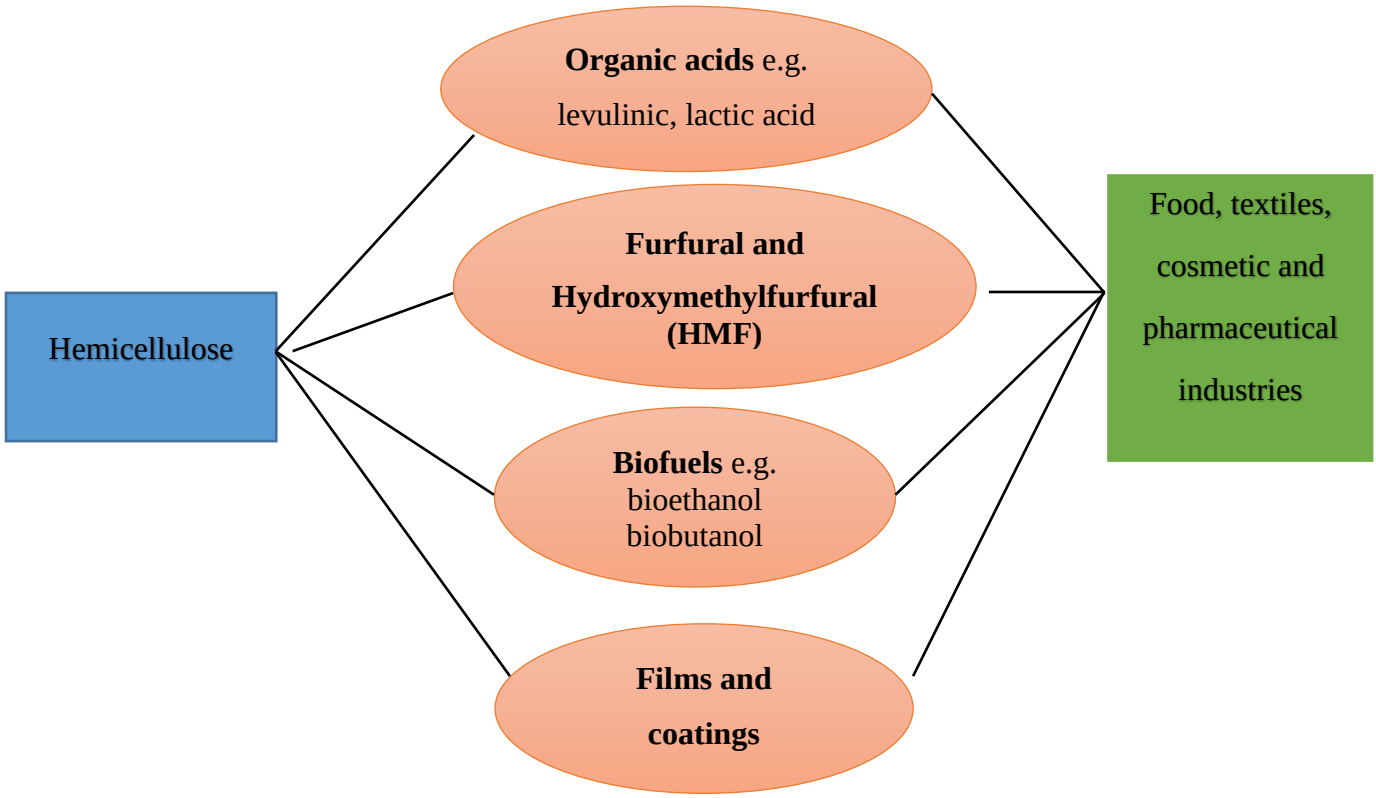

Figure 5: Potential hemicellulose applications

Another equally important aspect to consider when selecting a hemicellulose extraction method is the effect of the extraction conditions on the purity of the hemicellulose. The major impurity which hinders the utilisation of hemicellulose-based films as food packaging material is lignin. Lignin acts as a barrier to hemicellulose modification by processes such as acetylation, due to the lignin's resistance to chemical and enzymatic attack (Takkellapati *et al.*, 2018). Hemicellulose modification is inevitable as unmodified hemicellulose exhibits poor mechanical and barrier properties, thereby limiting its application as a food packaging material. Although hemicellulose with higher lignin content has been reported to be more hydrophobic and has better mechanical properties than those with lower lignin, further modification of the hemicellulose is still crucial (Rabetafika *et al.*, 2014). In fact other factors like the arabinose/xylose ratio and uronic acid content (UAC) of hemicellulose overshadow the ability of lignin to reduce the hydrophilicity of the films, hence, hemicellulose needs to be modified to meet the water resistivity and mechanical properties requirements for food packaging (Sternemalm *et al.*, 2008; Egüés *et al.*, 2013; Gordobil *et al.*, 2014). In addition, lignin content higher than 4% in hemicellulose has been reported to cause a brownish opaque appearance in hemicellulose-based films (Höije *et al.*, 2005; Geng *et al.*, 2018).

Opaque food packaging films are less appealing to consumers when compared to transparent colourless films. Therefore, efforts have been made to minimise the lignin content of hemicellulose films by bleaching the hemicellulose or by pre-treating the biomass prior to hemicellulose extraction (Höije *et al.*, 2005; Egüés *et al.*, 2014).

Some researchers have included a bleaching step with hydrogen peroxide, or sodium chlorite to reduce the hemicellulose lignin content, thereby improving the appearance of the hemicellulose films (Egüés *et al.*, 2014). Sodium chlorite-based bleaching has proven to remove more lignin (33%) from hemicellulose when compared to hydrogen peroxide bleaching (22%); however, the use of the above-mentioned oxidising agents is considered environmentally unfriendly (Park *et al.*, 2015). More environmentally friendly procedures such as pre-treating biomass before hemicellulose extraction, have been developed to replace the bleaching process. Processes such as organosolv pre-treatment have been reported to remove up to 67% of the total lignin in hemicellulose (Höije *et al.*, 2005). Incorporation of pre-treatment stages before hemicellulose extraction has evidently also been done to facilitate the recovery of high yields of hemicellulose. Multiple step extraction processes which include combining mechanical, biological and chemical pre-treatment methods have significantly improved both hemicellulose yield and purity from different biomasses (Egüés *et al.*, 2012; Vandenbossche *et al.*, 2014). This is supported by a comparison of the results of a single hemicellulose extraction (NaOH fractionation) by Fang *et al.* (2000) and a two-step fractionation (delignification with NaOH/hydrogen peroxide, hemicellulose extraction with KOH) by Sun and Sun's (2002). The hemicellulose yield increased from 50% to 91% based on the total hemicellulose in the biomass with an increase in the number of fractionation steps (Fang *et al.*, 2000; Sun and Sun, 2002).

More importantly, due to the ability of pre-treatment processes to change hemicellulose composition by removal of lignin and side chains (uronic acids and arabinose), they can diversify the utilisation of hemicellulose (Peng, Peng, Xu and Sun, 2012). Therefore, this study investigated the potential of wheat straw pre-treatment with ethanol and NaOH to improve the overall functionality of the hemicellulose in the food packaging environment. This involved optimising the biomass fractionation process with the aim of tailor making the hemicellulose composition to enhance hemicellulose acetylation, hydrophobicity and the mechanical properties and stability of hemicellulose-based films when in contact with food.

2.2.4. Biomass pre-treatment methods

Hemicellulose, lignin and cellulose are intertwined in biomass. Therefore, to effectively recover these biopolymers from biomass, pre-treatment methods are required to break bonds and separate the lignocellulosic material (Kumar and Sharma, 2017). Biomass pre-treatment processes, also

known as delignification processes (Figure 6), have been applied prior to hemicellulose extraction to reduce the recalcitrance of lignin, thus, freeing hemicellulose and ensuring its effective extraction (Farhat, Venditti, Quick, Taha, Mignard, *et al.*, 2017). Technologies that have been applied in pre-treating biomass can be classified as conventional and emerging pre-treatment methods (Bussemaker and Zhang, 2013; Sagar *et al.*, 2018).

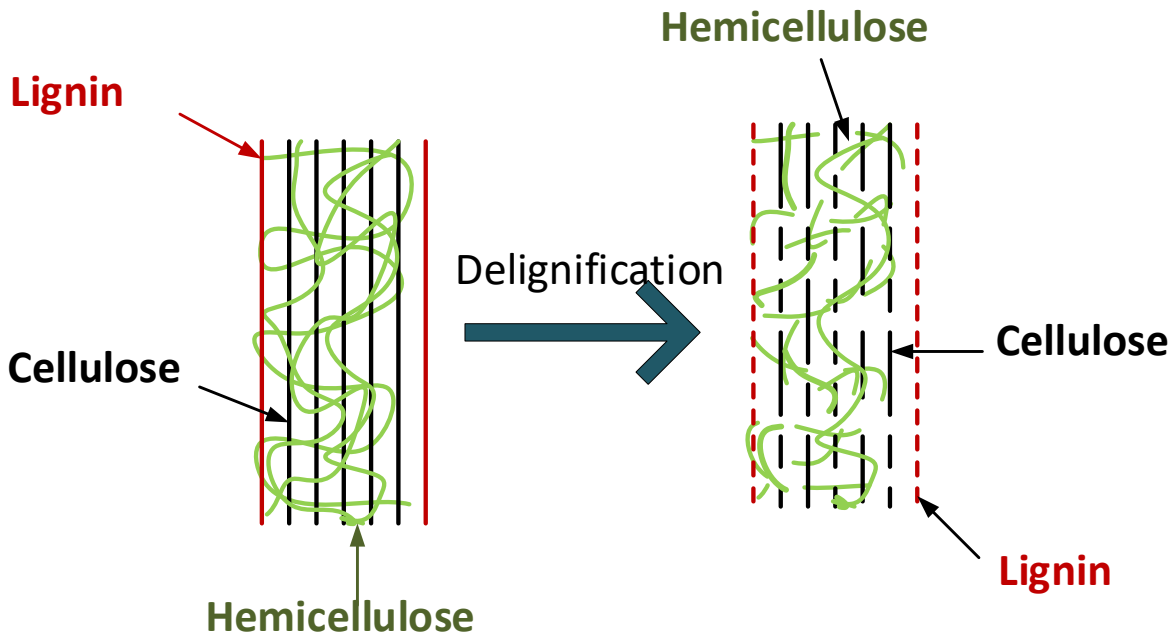

Figure 6: Schematic diagram for biomass delignification (adapted from Brodeur et al., 2011 drawn using Microsoft Visio)

There has been a growing interest in finding alternative pre-treatment methods to replace the existing conventional methods, namely, acidic, alkaline, mechanical and biological pre-treatment (Figure 6). New pre-treatment methods such as microwave assisted and ultrasound assisted pre-treatment (Hassan *et al.*, 2018) have been developed to curb the drawbacks of existing pre-treatment methods such as:

- The release of inhibiting agents which affect the effective extraction or further processing of hemicellulose
- Substantial loss of hemicellulose due to degradation
- The use of environmentally unfriendly chemicals that may end up polluting the environment if not properly disposed.

However, the conventional methods are still the preferred pre-treatment methods due to the high capital costs associated with implementing and operating the new technologies at industrial scale (Hassan *et al.*, 2018). Figure 7 shows the commonly utilised methods for delignification of biomass prior to hemicellulose extraction.

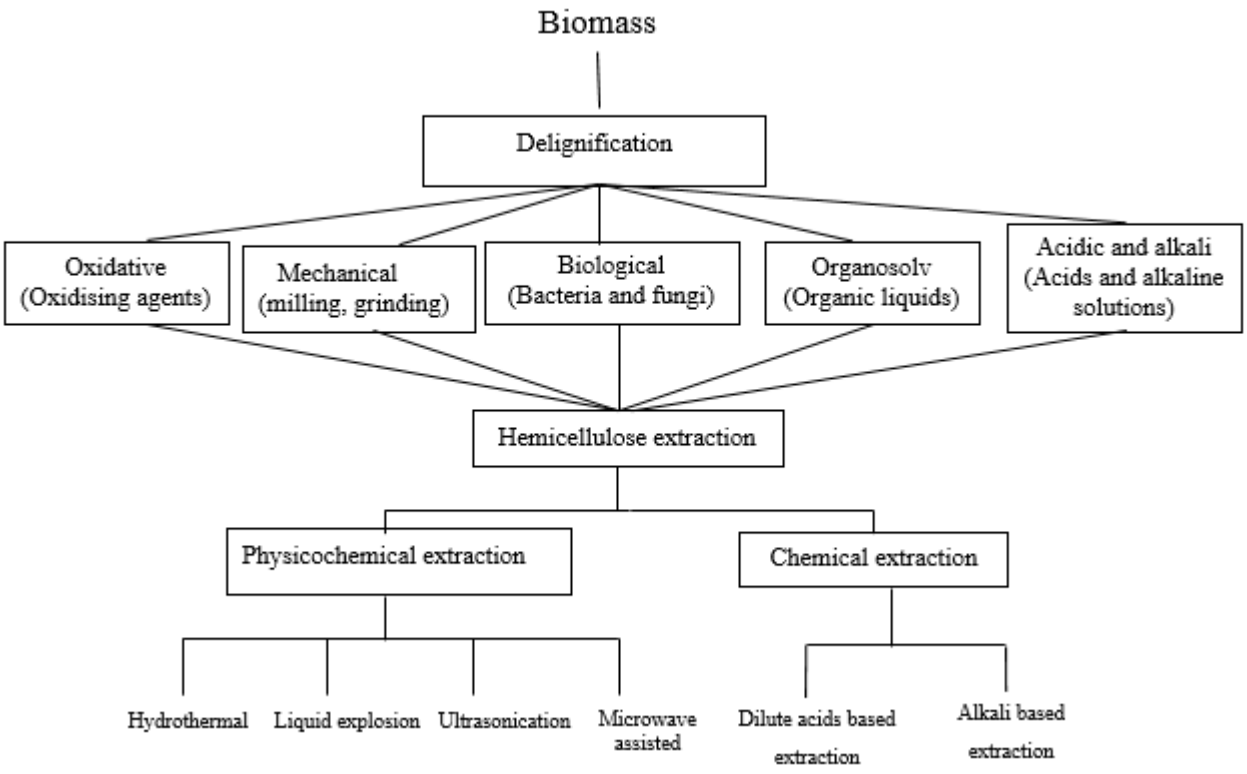

Figure 7: Biomass pre-treatment methods for hemicellulose recovery

2.2.5.1. Oxidative delignification process

Oxidative delignification involves the utilisation of oxidising agents such as sodium chlorite, hydrogen peroxide and oxygen to deconstruct biomass before hemicellulose extraction. The mechanism behind the delignification process involves the disintegration of the oxidising agents to form highly reactive hydroxyl groups. The hydroxyl radicals break the intermolecular bonds between hemicellulose and lignin in biomass, thereby freeing hemicellulose (Rabetafika *et al.*, 2014). According to Park *et al.*, (2015) oxidative delignification with sodium chlorite is most effective in removing lignin from woods while the use of hydrogen peroxide is the least effective (Park *et al.*, 2015). Reaction conditions normally used during oxidative delignification include high temperatures ranging from 125 ^{o}C to 300 ^{o}C and high pressures ranging from 0.5 to 20 MPa (Den *et al.*, 2018). Oxidative delignification is known to remove substantial amounts of lignin from biomass and enhances the yields of hemicellulose that can be recovered from biomass. The findings in the work by Rabetafika *et al.*, (2014) show that oxidative delignification removed up to 99.5% of the original lignin in pear pomace and led to the recovery of up to 80.2% of the hemicellulose (Rabetafika *et al.*, 2014). However, the hemicellulose recovered was in monomeric form. In addition, the delignification method can result in substantial loss of hemicellulose when the monomeric hemicellulose is further broken down to furfural. Geng *et al.* (2018) highlighted a loss of up to 38 % of the original hemicellulose content of biomass when sodium chlorite was used to

delignify switch grass (Geng *et al.*, 2018). Therefore, oxidative delignification may not be a cost-effective pre-treatment method for biomass prior to polymeric hemicellulose extraction.

2.2.5.2. Organosolv delignification

Organosolv delignification involves the breakdown of the β-O-4 linkages in the lignin structure, and bonds between lignin and other carbohydrates in the biomass leading to the solubilisation of lignin in the organic solution (Guragain *et al.*, 2016). Organosolv delignification of biomass has been carried out at temperatures ranging from 100 ^{o}C to 200 ^{o}C with organic solvents such as ethanol, ethylene glycol, methanol, glycerol, peracetic acid and formic acid (Cybulska *et al.*, 2017; Borand and Karaosmanoğlu, 2018; Kalogiannis *et al.*, 2018). The use of ethylene glycol and glycerol to delignify biomass is limited by the high boiling points of the solvents which makes it expensive to recover and to recycle the solvents after biomass pre-treatment (Guragain *et al.*, 2016). Organic acids (peracetic acid and formic acid) are corrosive in nature, hence, their use to pre-treat biomass is discouraged by the requirement for specialised vessels. Methanol and ethanol are considered the most effective solvents for fractionating biomass to recover hemicellulose, lignin and cellulose with minimal degradation (Borand and Karaosmanoğlu, 2018). However, when the biomass derivatives are to be used for food related applications, ethanol is preferred as it is considered to be safer than methanol (Farahmandazad, 2015). Several catalysts have been used to increase the effectiveness of the organic solvent-based delignification. NaOH, magnesium sulphate, calcium hydroxide and sulphuric acid are among the chemicals that have been used as catalysts during organosolv delignification (Guragain *et al.*, 2016; Borand and Karaosmanoğlu, 2018). Therefore, based on the fact that the hemicellulose extracted in this study will be used to develop food packaging material, ethanol-based organosolv delignification of wheat straw was carried out before hemicellulose extraction.

2.2.5.3. Mechanical pre-treatment

Mechanical pre-treatment methods such as grinding, milling and chipping reduce the particle size of biomass, thereby increasing the surface area for hemicellulose extraction. Milling and grinding of biomass achieves particle size ranging from 0.2 mm to 2 mm while chipping reduces particle size to between 10 mm and 30 mm (Kumar *et al.*, 2009). Mechanical pre-treatment is regarded as the least effective biomass pre-treatment method when compared to other pre-treatment methods and is normally coupled with other pre-treatment methods (biological, chemical) (Nauman Aftab *et al.*, 2019). Research findings by Kim *et al.*, (2019) support this fact. They observed an increase in hemicellulose yield of only 1.1% when milling severity was increased from 6000 rpm to 20 000 rpm. Furthermore, increasing the milling severity resulted in degradation of hemicellulose.

Combining mechanical pre-treatment with other treatment methods such as alkaline-based treatment has achieved hemicellulose recovery of up to 93.76% (Qu *et al.*, 2017; Kim *et al.*, 2019).

Mechanical pre-treatment methods are energy intensive, with the power consumption depending on the biomass type and the particle size required (Amin *et al.*, 2017; Baruah *et al.*, 2018). Softwoods such as agricultural residues (for example corn stover, needs 11 kWh/metric ton), require less energy during mechanical pre-treatment when compared to hardwoods (for example poplar chips require 118.5 kWh/metric ton). In addition, the smaller the particle size required, the more energy is consumed during pre-treatment (Baruah *et al.*, 2018). Therefore, it is important to control the mechanical pre-treatment method for effective breakdown of biomass at an economical energy consumption rate. In this study, wheat straw was milled to increase the surface area for organosolv delignification and hemicellulose extraction.

2.2.5.4. Biological delignification

Biological delignification is a biomass pre-treatment method which uses bacteria and fungi that target lignin (Brodeur *et al.*, 2011). Enzymes such as laccase and peroxidase which act upon lignin only in the biomass are released by the microorganisms. The breakdown of lignin increases the surface area of hemicellulose exposed to the extraction medium in the hemicellulose extraction stage (Kumar *et al.*, 2009). Therefore, higher yields of hemicellulose can be attained after delignification when compared to a single-step hemicellulose extraction process. Laccase (160 U/g) was reported to increase the specific surface area for hemicellulose extraction by up to 95.6%, resulting in a recovery of 88.7% of the hemicellulose from bagasse. A hemicellulose yield of only about 16.33% was achieved when hemicellulose was recovered without pre-treating the bagasse (Huang *et al.*, 2019). The advantages of biological delignification are that the microorganisms specifically target lignin without affecting the other biomass components and the procedure is environmentally friendly (reduces reliance on hazardous chemicals) (Anwar *et al.*, 2014). However, large scale pre-treatment of biomass using microorganisms is inhibited by the need of large dosages of costly enzymes and the long reaction times required for effective delignification (Saini *et al.*, 2015). Hence, biological delignification of biomass to recover large quantities of hemicellulose required for producing food packaging material at industrial scale is not a viable option.

2.2.5.5. Cavitation-based delignification

Cavitation based delignification of biomass involves the rapid build-up and collapse of bubbles in the biomass structure leading to biomass disintegration (Baxi and Pandit, 2012). The cavitation process is achieved by increasing temperature and pressure to up to 10^4 K and 10^3 bars, respectively, and rapidly decreasing these conditions to ambient conditions. The sudden change in temperature and pressure result in breaking bonds within the biomass. Delignification by cavitation

has been carried out using organic solvents (ethanol), acids (sulphuric acid), water and alkaline solvents (NaOH and potassium hydroxide) (Li *et al.*, 2010; Terán Hilares *et al.*, 2016). Cavitation delignification has been classified into two categories, namely hydrodynamic and acoustic cavitation (Madison *et al.*, 2017). Hydrodynamic cavitation involves the use of a constriction (venturi and orifices) to increase the pressure of the solvent flowing into the reactor. This leads to the formation of bubbles which consequently burst as the pressure of the liquid in the reactor declines, thus, causing biomass in the reactor to breakdown. Acoustic cavitation is carried out by propagating ultrasound waves through biomass. The waves form cavity regions where gas bubbles form and burst, thereby breaking down biomass. Comparing the two types of cavitation, hydrothermal cavitation is a more effective delignification method than acoustic cavitation. Baxi and Pandit (2012) reported that the rate of delignification by hydrothermal cavitation is 4-5 fold higher than that of acoustic cavitation (Baxi and Pandit, 2012). Although high hemicellulose yields (93.05%) are recovered using this technology, it is in monomeric form, hence limiting the application of the method in recovering hemicellulose for use in developing food packaging (Terán Hilares *et al.*, 2017).

2.2.5.6. Chemical pre-treatment

Chemical pre-treatment of biomass utilises alkaline and acids to deconstruct it, thereby increasing the surface area for hemicellulose extraction. Alkaline solvents such as NaOH, potassium hydroxide and acids such as hydrochloric acid and sulphuric acid have been used for pre-treating biomass before hemicellulose extraction (Brodeur *et al.*, 2011). Chemical pre-treatment has been reported as the most effective delignification method when compared to the other pre-treatment methods (Lee, Hamid, *et al.*, 2014).

The mechanism by which acid-based pre-treatment fractionates biomass involves the acid first disintegrating in water to form hydronium ions. The hydronium ions break down bonds between the hemicellulose, lignin and cellulose in biomass. The major drawback of the acid-based pre-treatment is that it causes depolymerisation of the hemicellulose as the hydronium ions also target the glycosidic bonds within the hemicellulose structure (Xiao *et al.*, 2017). In addition, this pre-treatment method uses strong acids, therefore, expensive corrosion resistant reactors are required. In order to minimise the degradation of hemicellulose, acid concentrations of less than 4% are used (Kumar *et al.*, 2009).

Alkaline-based delignification methods target the hemicellulose-lignin glycosidic ester bonds, boosting hemicellulose recovery from biomass (Lee, Hamid, *et al.*, 2014). Alkaline pre-treatment is normally operated at milder temperatures (as low as 25 ^{o}C), therefore it is less energy intensive when compared to acid based treatment (130 ^{o}C to 210 ^{o}C) (Badiei *et al.*, 2014). Additionally,

alkaline treatment of biomass results in minimal degradation of hemicellulose when compared to delignification with acids (Kim *et al.*, 2016). Therefore, alkaline-based pre-treatment of biomass is a more suitable and cost effective method for biomass delignification before hemicellulose extraction as it preserves the polymeric form of hemicellulose and does not require specialised reactors when compared to the acidic pre-treatment. Furthermore, by specifically targeting lignin, the alkaline pre-treatment method improves the purity of the recovered hemicellulose. Celebioglu *et al.* (2012) observed that hemicellulose extracted from corn waste (under the same temperature and time conditions) after alkaline pre-treatment had a purity of 40.2% while the hemicellulose recovered after acidic pre-treatment had a purity of 27.4% (Celebioglu *et al.*, 2012). Hence, in this study, alkaline-based delignification of wheat straw was found to be the most appropriate biomass pre-treatment method prior to recovering hemicellulose for developing active food packaging films.

2.2.5.7. Combination of pre-treatment methods

A combination of pre-treatment methods has been applied to counter the drawbacks exhibited by each individual pre-treatment method such as long reaction time and cost. The most commonly utilised pre-treatment combination is the physical pre-treatment method with either the chemical or biological pre-treatment methods (Lee, Hamid, *et al.*, 2014). Grinding or milling of the biomass has proven to effectively increase the biomass surface area for chemical and biological reactions (Brienzo and Figueiredo, 2016). More importantly, incorporating the physical pre-treatment reduces the quantities of chemicals and microorganisms required during the chemical and biological pre-treatment, respectively. Li *et al.* (2015) reported a reduction in the concentration of NaOH (from 8% to 4%) required to extract hemicellulose after combining the mechanical treatment and sodium hydroxide-based treatment of biomass (Li et al., 2015). Hence, environmental pollution associated with the utilisation of chemicals was minimised. Furthermore, combining pre-treatment methods has the potential to result in energy savings. Thangavelu *et al.* (2018) carried out a sequential delignification of corn cob using hydrothermal cavitation and enzymatic pre-treatment. They recorded a reduction in energy utilisation by 1.35 MJ/kg (Thangavelu *et al.*, 2018). Therefore, a combination of biomass pre-treatment methods has to be considered when designing cost-effective and sustainable biorefineries for agro residues. In this study, wheat straw was milled to reduce the biomass particle size, prior to alkali-catalysed organosolv pre-treatment and alkali-based hemicellulose extraction.

2.2.5. Hemicellulose extraction processes

Liquid explosion (Martin-Sampedro *et al.*, 2014; Boonterm *et al.*, 2016), dilute acid extraction, and alkali-based extraction (Palm and Zacchi, 2003; Tunc and Van Heiningen, 2008; Egüés *et al.*, 2012) are among the commonly used methods in recovering hemicellulose from biomass. However, these approaches rely heavily on the use of chemicals, hence technologies such as microwave-assisted extraction, ultrasound-assisted extraction, and hydrothermal extraction have been developed which use minimal or no chemicals (Peng, Peng, Xu and Sun, 2012). The conventional methods, however, still remain the most cost-effective and feasible methods for large scale hemicellulose isolation (Table 2). Comparing the aforementioned conventional hemicellulose isolation technologies, the alkali-based extraction method is the most preferred for extracting hemicellulose for formulating packaging material because it recovers high yields of polymeric hemicellulose. Svärd *et al.*, (2017) achieved a hemicellulose yield of 47% after using NaOH to extract hemicellulose from rapeseed and recovered only 5% of the hemicellulose with hydrochloric acid (Svärd *et al.*, 2017). On the other, hand liquid explosion degrades most of the hemicellulose into monomers as it is carried out at temperatures > 200 ^{o}C. Polymeric hemicellulose has proven to produce tougher and more elastic films as compared to monomers from degraded hemicellulose (Farhat, Venditti, *et al.*, 2017; Mendes *et al.*, 2017).

More importantly, alkaline solvents can cleave side branches present in hemicellulose during extraction, thereby altering hemicellulose composition (Naidu *et al.*, 2018). Removal of these side groups reduce hemicellulose solubility and improves hemicellulose-based films mechanical properties, a property which is important in formation of water-resistant food packaging films (Stepan *et al.*, 2012). These properties are essential for production of flexible and good barrier food packaging material comparable to LDPE based packaging. Therefore, alkali-based extraction of hemicellulose is an ideal biomass fractionation method in recovering hemicellulose to be used for packaging film formulation.The following section explains the different hemicellulose extraction methods in detail.

Table 2: Hemicellulose yields obtained from different extraction methods

Biomass	Number of stages	Extraction conditions	Hemicellulose yield (%)	Reference
Hardwood craft pulp	Single step	Reacting with water, at 80 ^{o}C for 30 min	0.49	Liu *et al.* (2018)
	Single step	Reacting with water at 50 ^{o}C for 30 min	0.61	Liu *et al.* (2018)
	Single step	Treatment with 4% NaOH at 80 ^{o}C for 30 min	7.72	Liu *et al.* (2018)
	Single step	Treatment with 4% NaOH at 50 ^{o}C for 30 min	6.5	Liu *et al.* (2018)
	Combination of 2 methods	Microwave treatment (500 W) + water extraction at 50 ^{o}C for 30 min	0.75	Liu *et al.* (2018)
	Combination of 2 methods	Microwave treatment (500 W) + water extraction at 80 ^{o}C for 30 min	1.53	Liu *et al.* (2018)
	Combination of 2 methods	Microwave treatment (500 W)+ 4% NaOH extraction at 50 ^{o}C for 30 min	9.25	Liu *et al.* (2018)
Corn cob	Combination of 2 methods	Delignification with potassium permanganate, three stage extraction with water, 2% NaOH at 90 ^{o}C for 2 h and then 60% ethanol/3% NaOH solution, at 60 ^{o}C for 3 h	77%	Ma *et al.* (2016)
Switch grass	Single step	Treatment with 10% NaOH at 50 ^{o}C for 3 h,	89.5	Farhat *et al.* (2017)
Catalpa wood	Single step	Hydrothermal treatment at 170 ^{o}C for 1 h	40.6	Gallina *et al.* (2018)
Wheat straw	Single step	Treatment with 10% NaOH, at 40 ^{o}C for 90 min,	20.1	Garcia, (2015)
Sugarcane bagasse	Single step	Treatment with 1 M NaOH, at 40 ^{o}C for 18 h,	55.5	Xu *et al.* (2006)

2.2.5.1. Liquid explosion extraction method

The liquid explosion method utilises chemicals like water and ammonia to fractionate lignocellulosic material at high temperatures (180-200^{o}C) (Stoffel *et al.*, 2017). During liquid explosion glycosidic bonds in biomass break down after being subjected to shear forces arising from liquid expansion at high temperatures. Organic acids such as acetic acid are liberated in the process, and these further facilitate the hydrolysis of glycosidic bonds in biomass. Catalysts such as sulphuric acid can be used to increase the rate of hemicellulose extraction. Stoffel *et al.* (2017) reported a hemicellulose yield increase from 69% to 90% when the sulphuric acid catalyst concentration was increased from 0.75% to 3% (Stoffel *et al.*, 2017). The major drawback of this

process is that a low yield of polymeric hemicellulose (32-67%) is obtained as some of the hemicellulose is degraded to monomers, furfural and 5-hydroxymethylfurfural (Martin-Sampedro *et al.*, 2014; Stoffel *et al.*, 2017). More importantly, the purity of the extracted hemicellulose is compromised by the by-products of hemicellulose degradation as well as cellulose, lignin and other plant material co-extracted with the hemicellulose at high temperatures (Palm and Zacchi, 2003). When compared to other extraction methods such as alkali treatment, liquid explosion has the advantage of requiring shorter extraction times (5 to 10 min) (Stoffel *et al.*, 2017). The operational residence time is kept as minimal as possible to reduce the loss of hemicellulose as a result of overexposing the biomass to high temperatures (Naidu *et al.*, 2018). Hence, the shorter reaction time and the ability of the process to break bonds in biomass, ensuring effective recovery of hemicellulose, makes steam explosion highly suitable for hemicellulose extraction especially if the hemicellulose end-use requires the monomers from hemicellulose (Rao et al 2016). However, utilisation of steam explosion for recovering hemicellulose for food packaging is limited as the polymeric form of the hemicellulose is required for film formation.

2.2.5.2. Dilute acid extraction method

Dilute acid extraction employs for example hydrochloric acid and sulphuric acid to hydrolyse biomass, thereby solubilising hemicellulose (Vena *et al.*, 2015; Svärd *et al.*, 2017). Hemicellulose extraction using dilute acids has been carried out at temperatures ranging from 120-160 ^{o}C for 30 min (Jeong *et al.*, 2010; Vena *et al.*, 2015). A combination of high temperatures and acidic conditions is known to degrade hemicellulose particularly hemicellulose that contain acetyl groups (for example O-acetyl galactoglucomannans). Acetyl groups in hemicellulose are easily converted to acetic acid which facilitate the hydrolysis of hemicellulose (Wayman, 2013). The hydrolysis of hemicellulose produces xylose and other sugar monomers which can further be hydrolysed to furfural (Vena *et al.*, 2015). Utilisation of dilute acids for hemicellulose extraction with the aim of producing films for food packaging, is limited by the acids being corrosive and by degradation of the hemicellulose to monomers. Gallina *et al.* (2018) reported that acid concentrations above 4% cause a complete degradation of hemicellulose in a biomass (Gallina *et al.*, 2018). Additionally there is a need for a neutralisation step, for the liquids and solids remaining after the acid based extraction. The neutralisation step contributes to the cost of hemicellulose extraction, thereby making the process expensive (Saha, 2003; Anwar *et al.*, 2014).

2.2.5.3. Hydrothermal extraction

Hydrothermal extraction involves the dissolution of hemicellulose in biomass by reacting the biomass with hot water at temperatures as high as 240 ^{o}C for up to 90 min (Krogell *et al.*, 2013; Gallina *et al.*, 2018; Santos *et al.*, 2018). Water is maintained in the liquid state during hydrothermal extraction by raising the reaction pressure to above 9 bars (Gallina *et al.*, 2018). The water molecules break down to form hydronium and hydroxyl ions at high temperatures. Hydronium ions act as catalysts in the hydrolysis of glycosidic bonds and covalent bonds in hemicellulose and hemicellulose-lignin linkages. In addition, acetyl groups released by hemicellulose during the extraction process further aid in the breaking of bonds in biomass (Farhat *et al.*, 2016; Xiao *et al.*, 2017). However, the prolonged exposure of hemicellulose to acidic solutions can degrade the polymeric hemicellulose to oligomeric and monomeric form. Additionally, acetyl groups are released into the extraction media as acetic acid when hydrolysis of hemicellulose occurs, further reducing the pH of the solvent (Wayman, 2013). Consequently, the highly acidic medium further breaks down the monomeric hemicellulose to furfural.

Hemicellulose yields of up to 90% have been realised after applying the hydrothermal technology to biomass (Rissanen *et al.*, 2016). Hydrothermal extraction is done at temperatures higher than 90 ^{o}C, which may result in the co-extraction of glucose with the hemicellulose. Therefore, additional purification steps are required for hemicellulose recovered by hydrothermal extraction (Rissanen *et al.*, 2016). Hemicellulose extracted by the hydrothermal process has lower lignin content when compared to that recovered by steam explosion. In addition, there is lower depolymerisation of hemicellulose as the oligomeric form of hemicellulose is recovered by hydrothermal extraction whereas steam explosion and dilute acids results in mostly the monomeric sugars of the hemicellulose being extracted especially when long reaction times are used (Farhat *et al.*, 2016). Xiao, Song and Sun (2018) reported that the hydrothermal extraction method has the advantage of being environmentally friendly (Xiao *et al.*, 2017). In addition, since no chemicals are added, highly specialised equipment such as reactors with high corrosion resistance normally utilised with strong acids, are not required (Gallina *et al.*, 2018). However, the major drawback of this extraction method is that it results in substantial degradation of the hemicellulose to monomers and oligomers (Modenbach, 2014; Farhat *et al.*, 2016). Therefore, this process may not be suitable for recovering hemicellulose for developing food packaging material.

2.2.5.4. Ultrasonication

Ultrasonication, also known as cavitation, is a hemicellulose extraction method whereby sound waves are propagated through biomass soaked in a solvent, thereby facilitating its fractionation. The mechanism of biomass hydrolysis involves formation of air bubbles in the solvent, which grow and rapidly burst (Naidu *et al.*, 2018). The process enhances solvent to biomass interaction, thereby increasing hemicellulose solubilisation and extraction from biomass. Ultrasonication recovers high yields of hemicellulose at short extraction times and low temperatures (Zu *et al.*, 2012). For example, a hemicellulose yield as high as 95.2% was obtained after ultrasonicating eucalyptus grandis for 30 minutes. However, increasing the ultrasonication time beyond 30 min led to hemicellulose degradation, lowering the yield to 82.3% (Xu *et al.*, 2018). The main drawbacks of ultrasonication are that it is energy intensive and can cause structural changes in the hemicellulose especially when ultrasonication is done for prolonged periods of time (Bussemaker and Zhang, 2013).

2.2.5.5. Microwave-assisted extraction

Microwave-assisted extraction of hemicellulose is among the new and greener technologies for hemicellulose extraction (Hassan *et al.*, 2018). The method involves propagating waves, with a frequency ranging from 0.3 to 300 GHz, through the biomass, in the process reducing the glass transition temperature of the biomass and increasing the temperature of the extraction medium. Two mechanisms, namely, autohydrolysis and chemical ions assisted hydrolysis, are responsible for breaking down biomass during microwave-based extraction of hemicellulose. The autohydrolysis process breaks down biomass with the aid of acetic acid released by the biomass. Chemical ions assisted hydrolysis utilises ions such as sodium ions from alkaline solutions, used as the extraction medium (Kurian *et al.*, 2015). This technology has been used to extract hemicellulose from biomass such as birch wood, bagasse and corn (Taherzadeh and Karimi, 2008; Yoshida *et al.*, 2010; Panthapulakkal and Sain, 2013; Kurian *et al.*, 2015). Temperatures as high as 220 ^{o}C and extractions times ranging from 2 min to 20 min have been used in microwave-assisted extraction (Rose and Inglett, 2010; Coelho *et al.*, 2014). At the above mentioned conditions, hemicellulose yields of up to 75.2% were achieved (Yoshida *et al.*, 2010). However, the hemicellulose recovered by microwave-assisted extraction was in monomeric form due to the high temperature, thereby eliminating the utilisation of the hemicellulose for packaging film production. Catalysts such as NaOH, citric acid and sulphuric acid have been used during microwave-assisted extraction to enable the extraction of hemicellulose at temperatures as low as 60 ^{o}C (Janker-Obermeier *et al.*, 2012; Chadni *et al.*, 2019). Hemicellulose extraction at low temperatures in alkaline conditions minimises the breakdown of hemicellulose to monomers. Chadni *et al.* (2019) supports this fact when they reported that reducing the temperature at which microwave-assisted extraction is carried out to

below 100 ^{o}C under atmospheric pressure using NaOH as catalyst minimised the degradation of hemicellulose (< 2% monomers) (Chadni *et al.*, 2019). The polymeric form of hemicellulose by the above mentioned method increases the potential utilisation of the hemicellulose in developing food packaging material. However, large scale extraction of hemicellulose using microwaves is limited by the complexity of the process. For instance, microwaves are not capable of penetrating large volumes of biomass, hence, can result in some spots in the biomass being overheated and some being unprocessed. Processes such as stirring to increase the effectiveness of the extraction process are inhibited by the lack of compatibility of the microwave-assisted extraction and materials used in developing stirring equipment (Veggi *et al.*, 2013).

2.2.5.6. Alkaline extraction method

Alkaline-based extraction works by cleaving α-ether linkages between hemicellulose and lignin and ester bonds between hemicellulose and hydroxycinnamic acids (ferulic acid and p-coumaric acid) (Peng, Peng, Xu and Sun, 2012). The chemicals that are used for alkaline extraction of hemicellulose from biomass include NaOH, lithium hydroxide, barium hydroxide, calcium hydroxide, ammonium hydroxide and potassium hydroxide (Ren *et al.*, 2007; Naidu *et al.*, 2018). Among the above mentioned chemicals, the most commonly used for hemicellulose extraction from lignocellulosic biomass are NaOH and potassium hydroxide. Sodium hydroxide based extraction processes have been reported to yield more hemicellulose from biomass when compared to potassium hydroxide based extraction processes (Peng, Peng, Xu and Sun, 2012).

Processes that use alkaline solvents are regarded as mild when compared to acid based processes and can be carried out in less expensive vessels. Additionally, alkaline based extraction of hemicellulose is effective in breaking the hemicellulose-lignin complex linkages with minimal hemicellulose degradation when compared to acid based extraction processes. The major drawback of alkaline extraction methods is the production of brown coloured xylan films (Figure 8), due to high residual lignin. These films can be less appealing to the food packaging industry as consumers are attracted by packaging through which they can see the packaged food (Brienzo *et al.*, 2009; Sabiha-Hanim and Siti-Norsafurah, 2012). In order to improve this property of hemicellulose, researchers have resorted to bleaching hemicellulose and optimising the biomass delignification/pre-treatment process prior to extracting hemicellulose (Egüés *et al.*, 2014; Gordobil *et al.*, 2014).

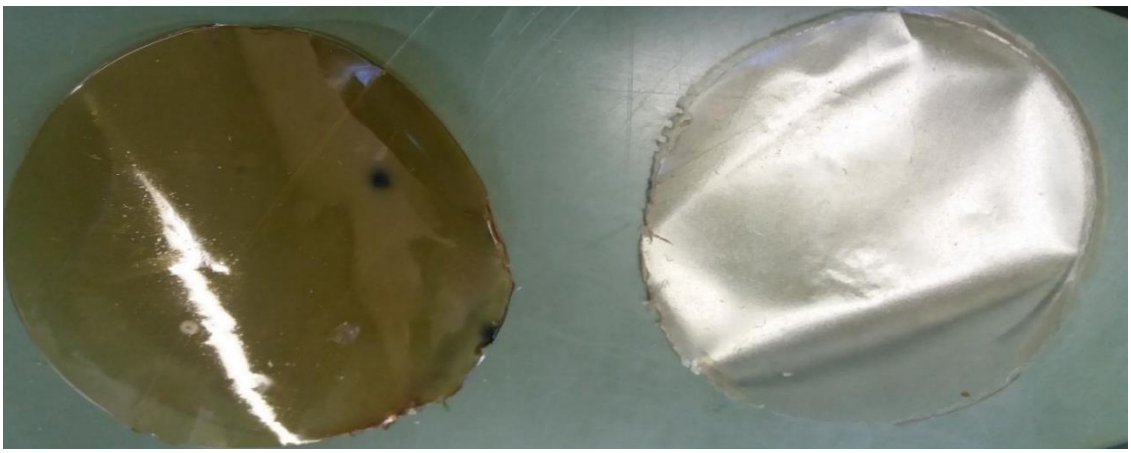

Figure 8: Films of hemicellulose extracted from wheat straw without delignification (Left) and after delignification (Right) (Mugwagwa and Chimphango- unpublished)

The bleaching step has the limitation of degrading hemicellulose to monomers and also it uses harmful chemicals that may not be suitable for food-contact packaging material (Singh *et al.*, 2008; Liu *et al.*, 2016). On the other hand, successive alkali-based pre-treatment of biomass has been reported to increase the purity of hemicellulose, thereby producing transparent films. Furthermore, alkaline-based pre-treatment is effective in producing linear hemicellulose chains with less branching, making the hemicellulose less soluble in water. A reduction in hemicellulose branching could be an effective method in increasing hydrophobicity of hemicellulose (Naidu *et al.*, 2018). Therefore, alkaline-based fractionation of biomass has the potential to recover hemicellulose suitable for developing food packaging films.

2.2.6. Hemicellulose modification for food packaging application

Utilisation of hemicellulose in food packaging is greatly hindered by its high affinity for water and poor mechanical properties when compared to petroleum-based food packaging (Farhat, Venditti, *et al.*, 2017). The extent to which hemicellulose is soluble in water is influenced by the side branches and acetyl group content of the hemicellulose (Sun *et al.*, 2010a). In order to minimise the solubility of hemicellulose in water, methods to reduce the side chains in hemicellulose were developed. The methods include using enzymes and chemicals to modify hemicellulose as well as blending the hemicellulose with other polymers when developing hemicellulose-based films (Table 3) (Chen *et al.*, 2015; Li and Pan, 2018; Rao *et al.*, 2019). More details on the strategies used to improve hydrophobicity and mechanical properties of hemicellulose-based films are presented in the subsequent sections.

2.2.6.1. Enzymatic treatment

The cleaving of side chains in hemicellulose with enzymes such as laccase, α-l-arabinofuranosidase, glucuronidase and galactosidase (Li and Pan, 2018) is the most commonly used method to enhance the hydrophobicity of hemicellulose (Hoije, Sternemalm, Heikkinen, Tenkanen, & Gatenholm, 2008; Kochumalayil & Berglund, 2014; Heikkinen *et al.*, 2013). Heikkinen *et al.* (2013) observed an improvement in moisture barrier properties of xylan films after the removal of arabinose side chains with enzymes (Heikkinen *et al.*, 2013). The findings of Kochumalayil and Berglund (2013) highlighted that the removal of galactose side chains by enzymatic treatment also reduced the hydrophilicity of xyloglucan films (Kochumalayil and Berglund, 2014). Xylan chains tend to entangle when side chains are removed. This phenomena is attributed to intermolecular bonding between the hemicellulose chains. Consequently, the hemicellulose chains become closely packed, reducing the penetration of water molecules through their structure, thereby minimising hemicellulose solubility in water (Li and Pan, 2018). Enzymatic treatment can be costly when modifying hemicellulose at a large scale, as cocktails containing the enzymes which can act upon arabinose and the different uronic acids present in hemicellulose will be required in large dosages. However, enzymatic modification has the advantage of targeting specific substrate without affecting the rest of the hemicellulose structure. Furthermore, the modification method is environmentally friendly as no chemicals are used during the process.
(Li and Pan, 2018).

Table 3: Hemicellulose modification for food packaging applications

Hemicellulose type	Modification	Objective	Results	Reference
Xyloglucan	Grafted with polyacrylic acid	To improve oxygen barrier properties	Lowered the xyloglucan films oxygen permeability from 2.16 to 0.25 $cm^3/\mu m/(m^2*d*kpa)$	Liu *et al*. (2019)
Xylans	Blended polyvinyl alcohol and chitin nanowhiskers	To improve mechanical and oxygen barrier properties	An improvement in tensile strength from 1.39 % to 19.80 %, reduction in oxygen transmission rate from 44.41 to 12.6 $cm^3/m^2 \cdot 24\ h \cdot 0.1$ MPa 40 °C 0% RH	Chen *et al.* (2015)
Quaternized hemicellulose	Added graphene oxide	To improve mechanical properties of the films	Tensile strength increased from 10.02 MPa to 32.85 MPa	Rao *et al.* (2019)
Quantenized hemicellulose	Added chitosan and montmorillonite	To improve mechanical properties of the films	Tensile strength increased from 57.8 MPa from 44.4 MPa	Chen et al. (2016)
AH	Reinforced with ACNC	To improve mechanical properties and hydrophobicity	WCA increased from 59^o to 82.1^o, Young's modulus from 293 MPa to 2241 MPa and tensile strength from 9 MPa to 67.30 MPa	Egüés, Stepan, Eceiza, Toriz, *et al.*, (2014)
Xylans	Reinforced with nanocellulose	To improve mechanical properties	Tensile strength increased from 11.9 MPa to 150.4 MPa and the Young's modulus increased from 735 MPa to 1721 MPa	Peng *et al.*, (2011)
Xylans	Acetylated to a DS of 1.8	To improve thermal stability of films	Film degradation temperature increased from 239 ^oC to 334 ^oC	Gordobil *et al.* (2014)

AH: acetylated hemicellulose; DS: degree of acetylation; ACNC: acetylated nanocellulose

2.2.6.2. Hemicellulose modification chemical-based methods

Modification methods such as grafting (Edlund and Albertsson, 2014), esterification (Ayoub *et al.*, 2013; Egüés *et al.*, 2014) and etherification (Nypelö *et al.*, 2016) have been applied to enhance the hydrophobicity and mechanical properties of hemicellulose-based films. The presence of two hydroxyl groups on the xylose structure of hemicellulose enables hemicellulose modification by the above mentioned methods (Hansen and Plackett, 2008; Sun *et al.*, 2010a)**.** The mechanism of operation of these modification methods involves substituting the hydroxyl groups in hemicellulose (Figure 9) with non-polar groups (acetyl groups, benzyl groups), thus, preventing the hemicellulose from forming hydrogen-based networks with water (Egüés *et al.*, 2014). Incorporating acetyl groups (acetylation) into biopolymers is a more favourable strategy in enhancing hydrophobicity and mechanical properties of biobased films for use as packaging material in contact with food than benzylation (Li and Pan, 2018). Benzyl groups are feared to be carcinogenic and the use of packaging material containing these chemical groups is discouraged by the International Agency for Research on cancer (IARC) (Salviano dos Santos *et al.*, 2015). The other advantages of acetylation vs modification processes such as grafting, silanation and corona treatment are that acetylation uses less toxic chemicals, cheaper chemicals and does not require expensive equipment (Lepetit *et al.*, 2017). Therefore, in this study, hemicellulose and nanocellulose were acetylated prior to developing hemicellulose-based active packaging material.

2.2.6.2.1. Acetylation process

The acetylation process uses chemicals such as acetic anhydride, succinic anhydride, and maleic anhydride to substitute the hydroxyl groups in hemicellulose with acyl groups (Huber and Petit-conil, 2011; Akkus *et al.*, 2018). To effectively acetylate polymers catalysts are introduced to activate the carbonyl groups in the acetylating medium (Sun *et al.*, 1999; Ren *et al.*, 2007, Belmokaddem *et al.*, 2011; Egüés, Stepan, Eceiza, Toriz, *et al.*, 2014). The most commonly used catalysts for acetylation, namely, pyridine and N,N-dimethylformamide (DMF) are toxic and cannot be used in processes for production of food packaging. The proposed method in this study utilises sulphuric acid as a catalyst. Sulphuric acid has previously been utilised as a catalyst when acetylating starch (Whistler *et al.*, 2012). The United States Food and Drug Administration (FDA) has approved the use of sulphuric acid in food-based applications (ICF International, 2012).

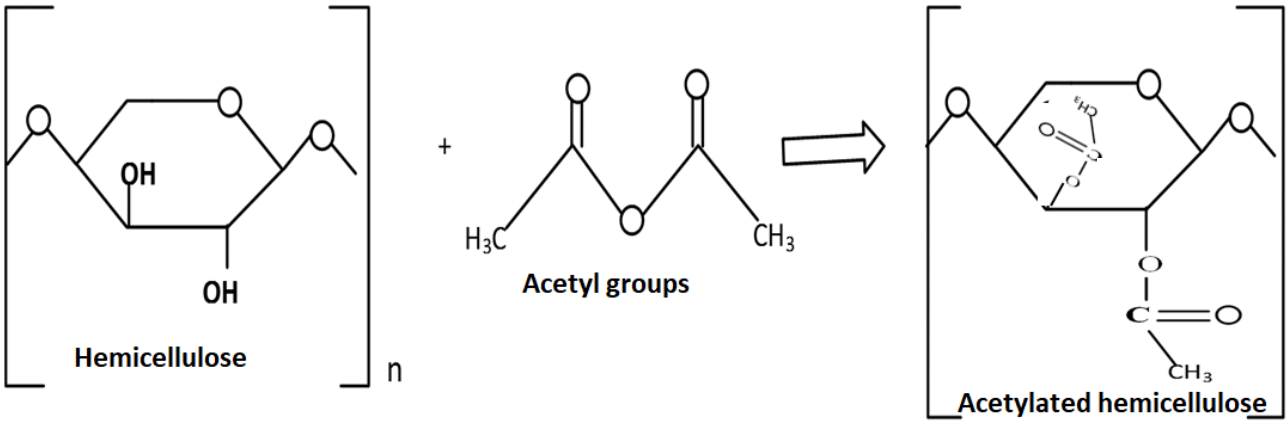

Figure 9: Schematic diagram of hemicellulose acetylation (drawn using Microsoft Visio)

In addition to reducing moisture sensitivity, acetylation improves the flexibility, thermoplasticity and resistance to acidic hydrolysis of the biopolymer based films (Gröndahl *et al.*, 2003; Xiaowen Chen *et al.*, 2012). Egüés *et al.* (2014) observed that the Young's modulus, flexibility and strength of hemicellulose-based films increased by 100%, 13% and 26% respectively after acetylation. Furthermore, the acetylated hemicellulose-based films exhibited thermal resistant behaviour suitable for thermoplastic processing. The thermal stability of the films improved from 220 ^{o}C to 375 ^{o}C after acetylation (Egüés *et al.*, 2014).

2.2.6.3. Blending of hemicellulose with other materials

Hemicellulose films have poor mechanical and barrier properties, hence, they require reinforcement material, coatings and plasticizers if they are to be used as food packaging material (Shimokawa *et al.*, 2015). The mechanical properties and hydrophobicity of hemicellulose films have been enhanced by incorporating other polymers such as chitosan, cellulose, nanocellulose, polyvinyl alcohol (PVA) and acrylamide (Saxena *et al.*, 2009; Peng *et al.*, 2011; Chen *et al.*, 2015; Chen *et al.*, 2016; Farhat *et al.*, 2016). Among the aforementioned polymers, nanocellulose is the most commonly used reinforcement for hemicellulose. Nanocellulose is particularly compatible (measured by comparing mechanical properties of the unreinforced and reinforced film) with deacetylated xylans (Boujemaouit et al., 2017; Pawar *et al.*, 2013). The abundance of hydroxyl groups in both the hemicellulose (with no acetyl groups) and nanocellulose structure facilitate hydrogen bonding between the two polymers (Xu *et al.*, 2004; Chakrabaty and Teramoto, 2018). Biomass pre-treatment methods such as dilute acid (for example sulphuric acid), alkali-based (for example sodium hydroxide) and enzymatic (carbohydrate esterase) treatment of biomass have be applied to deacetylate hemicellulose prior to extraction to improve its compatibility with nanocellulose (Peng, Peng, Xu and Sun, 2012).

Similar to hydrophobic matrices such as LDPE, poly (vinylacetate) and PCL, the utilisation of nanocellulose as a reinforcement material for AH films is hindered by its hydrophilic nature. Modification methods such as acetylation and polymer grafting have been proposed to reduce the hydrophilicity and increase the fibre-matrix interaction (derived from the reinforcing efficiency values of a fibre in a matrix), thereby improving the mechanical properties of the composite (Ansari *et al.*, 2015; Farhat *et al.*, 2018). For example, Ansari *et al.* (2015) observed that reinforcing poly vinylacetate with modified nanocellulose greatly improved the tensile strength of the composite by a factor of six. An improvement in AH film properties was also observed when these films were reinforced with ACNC. However, research on reinforcement of AH with ACNC is still limited to using ACNC with a low DS (0.54) (Ansari *et al.*, 2015). There is potential in further improving the properties of AH/ACNC films by increasing the DS of ACNC beyond 0.54.

Other than adding reinforcing material to packaging material, applying hydrophobic coatings on films can reduce the surface area of the film in contact with the environment, thus, improving the barrier properties of the films (Zhou *et al.*, 2013). Biodegradable plastics like PCL have found use as coatings on petroleum-based films such as polypropylene (Rešček *et al.*, 2018). PCL has been blended with biopolymers such as chitosan and starch (Joseph *et al.*, 2011; Ali Akbari Ghavimi *et al.*, 2015). Research on blending hemicellulose with PCL is still limited to the work by Fahart *et al.* (2018). Fahart *et al.* (2018) reported an improvement in mechanical and hydrophobicity of hemicellulose films after grafting hemicellulose with PCL. The co-polymerisation of hemicellulose with the PCL resulted in an increase in hydrophobicity of hemicellulose based films to 81^{o} from 61^{o} (Farhat *et al.*, 2018). Despite reports by Pawar *et al.* (2013) that hemicellulose acetylation improves the interaction of hemicellulose with plastics, the lamination of hemicellulose with PCL has not yet been done. Therefore, this study investigated the potential of PCL to laminate AH-based films with the aim of improving the films' hydrophobicity and mechanical properties.

2.2.7. Hemicellulose based films production process

The properties of hemicellulose-based films depend on the interaction of the film forming components with the solvent used for developing the films and the conditions applied to dry the films (Erdemir, 2015). The two most commonly used methods for thin film production in industry are solvent casting and hot melt extrusion (Particle Sciences, 2010; Quiroz-Castillo *et al.*, 2015). Several researchers have evaluated the effectiveness of the two film forming methods and concluded that the best method for producing hemicellulose-based films is the solvent casting method (Sabiha-Hanim and Siti-Norsafurah, 2012; Gordobil *et al.*, 2014; Erdemir, 2015). The criteria for selecting the solvent casting method over the extrusion method are highlighted in Table 4.

Table 4: Comparison of solvent casting method and hot melt extrusion method

Solvent casting method	Hot melt extrusion method
Lower temperatures are used therefore, suitable for heat sensitive film material	Thermal degradation of film ingredients due to high temperatures
The slow evaporation of solvents results in uniform films	The presence of volatile solvents in film ingredients results in the formation of voids in films causing the films to be non-uniform
Films may contain residual solvent of which some of the solvents maybe flammable	No residual solvents in films due to non-use of solvents or if solvents are added, the high operational temperature during extrusion removes all the solvents

2.2.7.1. Solvent casting method

Solvent casting is a method used in formulating thermoplastic films by drying the film solution in a mould, thereby forming a film with the shape of the mould. Film material is mixed with solvents such as water and chloroform. The mixture, also known as film dope is deposited onto a moving plate which passes through drying equipment such as an oven (Figure 10). Evaporating the solvent results in a continous film sheet (Particle Sciences, 2010; Amin *et al.*, 2015). The selection of a solvent and drying method to use during solvent casting is influenced by the solubility of the filmmaking components in the solvent and the flash point of the solvent (Buchanan *et al.*, 2003). In the case of hemicellulose-based films, the choice of solvent depends on the hydrophobicity of the hemicellulose. For example, hemicellulose from wheat straw (arabinoxylans) are hydrophilic, hence aqueous solutions are used for casting the films (Shimokawa *et al.*, 2015; Huang *et al.*, 2018). However, when hemicellulose is modified by processes such as acetylation to reduce hydrophobicity, water can no longer be used as a solvent. This is because acetylated hemicellulose becomes less soluble in water and more soluble in organic solvents with increasing DS. Therefore, organic solvents such as chloroform and dimethyl sulfoxide are used in developing acetylated hemicellulose-based films using the solvent casting method (Xu *et al.*, 2010; Fundador *et al.*, 2013; Zweckmair *et al.*, 2014).

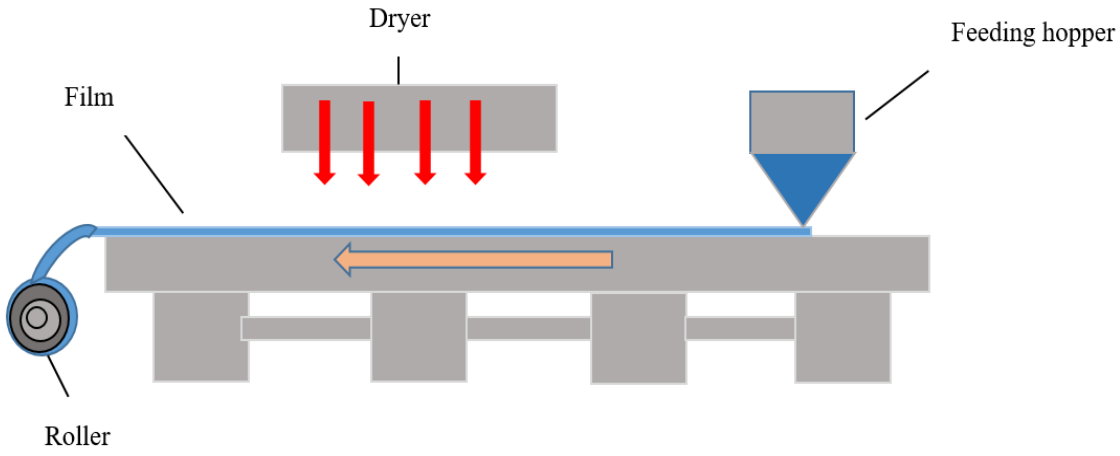

Figure 10: Schematic diagram of the solvent casting film system (Particle Sciences, 2010)

2.2.7.2. Hot melt extrusion method

Hot melt extrusion is a film making process that involves melting dry film ingredients in a cylindrical barrel that has one or two rotating screws. A flat extrusion die (Figure 11) then presses the molten material into films with different shapes and thickness. Examples of products which have been made using this method include plastic bags and pipes (Particle Sciences, 2010; Maniruzzaman *et al.*, 2012). Extrusion temperature and type of film forming material significantly influence the properties of the extruded film and consequently its end-application (Maniruzzaman *et al.*, 2012). Hemicellulose-based films have also been developed by extrusion. Erdemir, (2015) developed hemicellulose films using both the solvent casting and extrusion method. He reported that films formulated from cotton stalk hemicellulose extruded at higher temperatures (90 ^{o}C) had better mechanical properties (tensile strength-70.9 MPa, Young's modulus-1187 MPa) when compared to those formed at lower temperatures (75 ^{o}C) (tensile strength-65.6 MPa, Young's modulus-1024 MPa). However, comparing the extruded cotton stalk hemicellulose films to solvent casted films, the solvent casted films had higher tensile strength (91.5 MPa) than extruded films (72 MPa) (Erdemir, 2015).

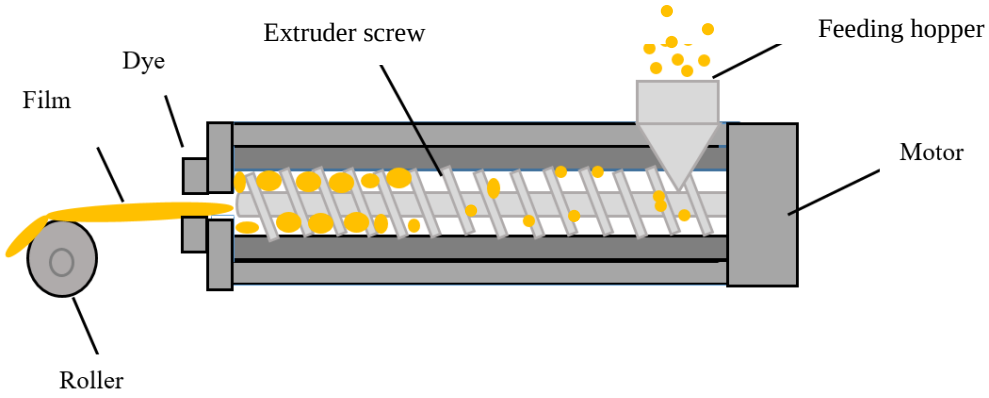

Figure 11: Schematic diagram of the film extrusion system (Particle Sciences, 2010)

2.3. Active food packaging

Recent research has devised mechanisms to enhance food packaging material so as to not only have good barrier properties but also to retain food quality and give information on whether food has been exposed to undesirable conditions. A packaging material with the above-mentioned properties is called an active packaging material (López de Dicastillo *et al.*, 2016; Nerin *et al.*, 2016; Dou *et al.*, 2018). Biodegradable (for example starch and pectin-based films) and non-biodegradable (for example LDPE) active packaging has been developed by incorporating synthetic and biobased sensors, antioxidants, and antimicrobial agents (Table 5) in the food packaging material (Espitia *et al.*, 2014; Suriyatem *et al.*, 2018; Gilakhakimabadi *et al.*, 2019, Zhong and Xia, 2008) Due to the awareness of the implications of artificial antioxidant compounds, the food packaging industry is now moving towards the use of natural bioactive compounds such as plant

derived oils and polyphenols, and animal-based bacteriocins and enzymes (Adilah *et al.*, 2018; Rababah *et al.*, 2004; Ravichandran *et al.*, 2011). The two common mechanisms of operation of active food packaging are to release active compounds into food in response to external stimuli (controlled release) and to change colour with changes in food storage conditions (temperature-time indicator) (Ghaani *et al.*, 2016). Packaging material with controlled release and temperature-time indicators can be strategies in increasing the shelf life of packaged food. A detailed description of temperature-time indicators and active packaging material is given in chapter 6 and 7.

Table 5: Active packaging materials and their properties

Film type	Active compound	Quantity of active compounds added	Active properties	Reference
Protein	Mango kernel extract	5%	89% antioxidant activity	Maryam Adilah *et al.* (2018)
Gelatin-sodium alginate	Tea polyphenols	2%	90% antioxidant activity	Dou *et al.* (2018)
Chitosan	Pomegranate rind powder extract	2%	75% antioxidant activity	Qin *et al.* (2015)
Chitosan/ polyvinyl alcohol	Red cabbage anthocyanins	25 %	Time-temperature indicator	Pereira *et al.* (2015)
Polylactic	Catechin	5%	Controlled release over time	Arrieta *et al.* (2014)
Nanocellulose fibre coating	Caffeine	7.5%	Controlled release with time	Lavoine *et al.* (2016)
Starch	Propolis	10%	Antimicrobial properties	Suriyatem *et al.* (2018)

2.3.1. Food packaging with controlled antioxidant release

Active food packaging materials with controlled antioxidant release are designed to ensure that active compounds get into the food in desired quantities and when required. The rate of releasing the active compounds should increase when microbiological organisms or oxidative radicals that cause food spoilage increase in packaged food (Dias *et al.*, 2018). Microbial growth and oxidative reactions in food, are facilitated by an increase in food storage temperature beyond the optimum, therefore a packaging material capable of releasing higher dosages of antioxidants with increasing temperature will be able to eliminate microbials and free radicals or minimise their quantities, thereby potentially preventing food spoilage (Duan *et al.*, 2013). Biobased films such as polylactic acid films, pectin films and protein films containing potassium sorbate, eugenol, tocopherols, nanotubes, and polyphenols as active compounds, were developed and the antioxidant release by the films into food was determined (López de Dicastillo *et al.*, 2016; Yu *et al.*, 2017; Dias *et al.*, 2018). Synthetic films such as LDPE have also been doped with antioxidants such as quecertin and tocopherols. Different antioxidant release profiles were obtained for the above-mentioned films indicating that the rate of antioxidant release is influenced by the type of packaging material and antioxidant as well as the interaction of the packaging material and the antioxidant.

The rate of release of the antioxidants of biobased films has been reported to be generally higher than from LDPE. For instance LDPE released less than 5% of antioxidants after exposure to a food simulant at 40 ^{o}C for 50 days. However, biobased films can release up to 100% of the antioxidants in less than 2 days (Xi Chen *et al.*, 2012; Yu *et al.*, 2017). The utilisation of LDPE films as active packaging films for perishable food is limited by the inability of LDPE to quickly release the

antioxidants. Therefore, biobased films have potential to replace LDPE films for packaging perishable food. On the other hand, the biobased controlled release capability has been studied using the fatty simulant and dry foods only as they are soluble in aqueous solutions. There is a need to modify biobased films to broaden their application as active packaging.

Hemicellulose application as an active packaging with controlled release of antioxidants has not yet been researched. Previous research has focused on instilling active properties in hemicellulose for biomedical purposes (Sun *et al.*, 2013; Gao *et al.*, 2016). In these studies, the potential of hemicellulose-based hydrogels to release encapsulated drugs has been the main goal of the research. For example, Gao *et al.* (2016) revealed that hemicellulose hydrogels can be used as pH/temperature indicators for controlled drug release (Gao *et al.*, 2016). In addition, the modification of hemicellulose to increase hydrophobicity prior to developing the active packaging can possibly widen its application.

2.3.2. Temperature-time indicator

Temperature-time indicators are used to detect and show if food has been exposed to temperatures not favourable for food storage. In addition, the indicator can show how long the food has been packaged for (Yousefi *et al.*, 2019). Plant extracts, microorganisms and dyes have been used as temperature-time indicators (Ghaani *et al.*, 2016). Most indicators operate by changing colour as a result of reactions facilitated by an increase in temperature (Yousefi *et al.*, 2019). Anthocyanins are among the promising plant-based temperature-indicators that will soon find application in the food packaging industry. These plant pigments are soluble in water and mainly found in fruits, vegetables and grains which are blue, purple or red in colour (Fang, 2015). Various authors have developed temperature-time indicators by incorporating the anthocyanins into biocomposite matrixes such as chitosan and starch (Maciel *et al.*, 2014; Pereira *et al.*, 2015). One of the most significant findings of Maciel *et al.* (2012) was that anthocyanins changed colour irreversibly when temperature increased from 40 ^{o}C to 70 ^{o}C (Maciel et al. 2012). Additionally, anthocyanins have been used to develop pH-time indicators due to their sensitivity to pH. In acidic conditions anthocyanins are pink, in neutral conditions they are blueish green and in basic conditions are violet. Pereira *et al*. (2015) was able to develop a pH-time indicator which detected milk spoilage with time. The anthocyanin-based indicator changed colour from dark grey to dark pink pH of the milk changed from 6.7 to 4.6 (Pereira *et al.*, 2015).

Anthocyanin composition influences the colour of the anthocyanins as well as their response to changes in temperature with time (Sipahli *et al.*, 2017). Red cabbage has been extensively studied as a source of anthocyanins for developing temperature-time indicators. This is because of the abundance of anthocyanins in red cabbage. However, the use of red cabbage as a feedstock for

anthocyanins for developing indicators competes with human food consumption. On the other hand, mango peels of the red coloured cultivars are discarded as waste in the mango fruit processing industry. Mango peel anthocyanins have only been identified and characterised, but their application as indicators is lacking. The main anthocyanin type in red cabbage is cyanidin-3-O-glucoside, however, mango peel anthocyanins have peonidin-3-O-glucoside, cyanidin 3-O-glucoside and peonidin-3-galactoside (Berardini, Fezer, *et al.*, 2005; Rees et al. 2012). Therefore, there is need to investigate the application of mango anthocyanins as indicators.

2.3.3. Sources of bioactive compounds

Bioactive compounds have been extracted from waste emanating from fruits (mangoes, lemons and oranges), vegetables (spinach, cabbage) and cereals (bran husk) (Sagar *et al.*, 2018). Among the aforementioned sources of bioactive compounds, fruit waste is an attractive source of polyphenols for active food packaging. Based on research done by Kahkonen and co-workers, (1999) fruit waste has a higher polyphenol content (ranging from 28.7 mg/g Gallic acid equivalent (GAE) to 50.8 mg/g GAE), followed by vegetables (0.4-6.6 mg/g GAE) and then cereals (0.2-1.3 mg/GAE) (Kahkonen, Hopia, Heikki, *et al.*, 1999). Moreover, fruit waste such as seeds and peels produced during fruit waste processing is currently discarded without further processing to recover valuable compounds (Serna-Cock *et al.*, 2016; Arora *et al.*, 2018). Therefore, the fractionation of fruit waste to recover bioactive compounds is a strategy to increase the value potential of the waste.

Mango peels have gained popularity as a source of polyphenols, although the polyphenol content of the mango peels is relatively lower than that of other fruit peels (Kahkonen, Hopia, Vuorela, *et al.*, 1999; Balasundram *et al.*, 2006; Rojas *et al.*, 2015). This is mainly due to the mango polyphenols having a higher antioxidant activity when compared to other citrus peels (Kahkonen, Hopia, Heikki, *et al.*, 1999). Maniyan et al. (2015) analysed the antioxidant level of 10 different fruit peels by estimating the reducing power percentage. The results showed that mango peels and pomegranate had the highest reducing power percentage (0.57%) followed by grapes (0.53%) and apple peels (0.5%) (Maniyan et al., 2015). The high antioxidant activity of the mango peel polyphenols is due to a collective contribution of all the polyphenols in the peel and not due to the abundance or presence of a certain polyphenol component type (Berardini, Knödler, *et al.*, 2005; Masibo and Qian, 2008). Thus, there is no need for further processing of the polyphenols to isolate a certain polyphenol type. More importantly, there are limits to the quantities of additives allowed in packaging hence bioactive compounds with higher activity at lower concentrations are desirable (Jamshidian *et al.*, 2013). Furthermore, research by Berardini, Knödler, *et al.*, (2005) showed that the mango peel polyphenol extract had a higher antioxidant capacity than standard mangiferin and quercetin, coming to the conclusion that it is the combined contribution of various polyphenols in

the extract which results in high antioxidant activity (Berardini, Knödler, *et al.*, 2005). Therefore, there is a need to optimise the mango peel fractionation process, focusing on recovering an extract with a high antioxidant activity.

Other than polyphenols, mango peels, particularly, the red-coloured cultivars, also contain anthocyanins and pectin which can also be utilised in developing active packaging (Kermani *et al.*, 2015; Rojas *et al.*, 2015). Mango peels contain approximately 10-15% pectin and 360–565 mg/100g anthocyanins (Kauser *et al.*, 2015; Banerjee *et al.*, 2016). Mango peels can therefore be used as a one stop feedstock for these products. However, application of the commonly used fractionation methods, whereby a single product is extracted while discarding the residue, underutilises the waste (Yates *et al.*, 2017). Despite the three products having different optimum extraction conditions, there is a need for a sequential extraction process to recover the three products. In a sequential extraction process each extraction stage affects the properties of products to be recovered in downstream processes. Hence, optimising the sequential extraction of anthocyanins, polyphenols, and pectin from mango peels while taking into consideration properties required in developing active packaging material is essential.

2.3.4. Integration of active compounds into packaging films

Changes in food quality during storage and distribution are a major concern for the food industry, as this can have detrimental effects on the health of consumers. These changes can be due to oxidation of food components such as lipids and microbial growth in the packaged food (López de Dicastillo *et al.*, 2016). Oxidative reactions can result in development of toxic compounds, degeneration of important nutrients causing food colour and flavour loss (Ganiari *et al.*, 2017). The approaches that have been adopted to reduce food spoilage involve adding antioxidants directly to food or to food packaging material (Jamshidian *et al.*, 2013). The addition of antioxidant compounds to packaging material (by dip spraying and coating the packaging material) has been highly recommended by previous researchers as antioxidants can be instantly deactivated by the food components if added directly to food (Helal *et al.*, 2012; Jamshidian *et al.*, 2013; Malhotra *et al.*, 2015). Moreover, agents of food spoilage accumulate on the surface of food, therefore, adding the antioxidants directly to food may unnecessarily increase the concentration of additives in food (Dias *et al.*, 2018). Therefore, packaging material with controlled release of antioxidants is a preferred option ensuring just in time delivery of the antioxidants to food (Uzunlu and Niranjan, 2017) .

Integration of polyphenols into biopolymers such as starch, chitosan and cellulose has been carried out successfully. However, polyphenols are known to disturb the structural orientation of the films, thereby affecting the films' barrier and mechanical properties (Cha and Chinnan, 2004; Shit and Shah, 2014). Contradictory results have been reported in the literature on the effect of the

polyphenols on film properties. Adilah *et al*. (2018) reported an improvement in the Young's modulus (from 11.25% to 12.64%) and tensile strength (1.82 to 7.74%) upon adding 5% mango kernel extract to protein-based films. In addition, the solubility of the protein-based films in water decreased from 33% to 22% (Adilah *et al.*, 2018). This, therefore, suggests that the antioxidants do not only prevent microbial growth in packaged food but can improve the mechanical properties of the films. However, Tan *et al.* (2019) observed a reduction in mechanical properties in starch films (for example tensile strength reduced from 27 MPa to 21 MPa) upon adding polyphenols. This, therefore, proves that different types of packaging material behave differently upon adding antioxidants. The effects of mango peel polyphenols on the properties of hemicellulose-based films have to be studied and not assumed based on the properties of a different material. Hence, before the hemicellulose-based films with mango polyphenols are to be used as an active packaging material for food packaging, the mechanical properties of these films need to be evaluated.

2.3.5. Testing of antioxidant release by active food packaging

The rate of migration of film material into packaged food with time is an aspect to consider when choosing a packaging material for a specific food type. An ideal packaging material should not react with packaged food as this may lead to food contamination (Azeredo *et al.*, 2017). Additives and monomers of the packaging material with low molecular weight have a tendency to migrate into food because they are not tightly bonded to the matrix of the material (Marsh and Bugusu, 2007). Nerin *et al.* (2016) has attributed the migration of additives in food packaging material into food to the four processes highlighted in Figure 12 (Nerin *et al.*, 2016). These processes are governed by two thermodynamics theories. The first theory is a mass transfer law, denoted by the diffusion coefficient, which state that particles diffuse from a region of high concentration to a region of low concentration. The second theory, known as the partition coefficient, describes the distribution of the migrant in the packaging material and in the food at equilibrium (Nerin *et al.*, 2016).

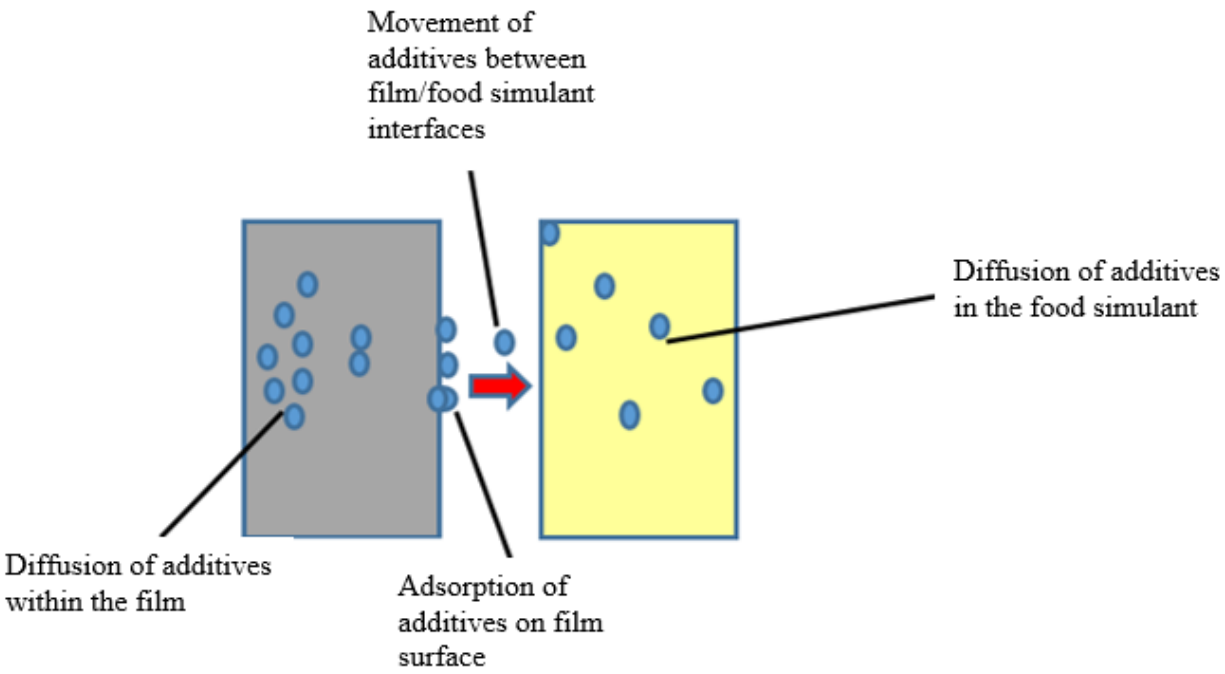

Figure 12: Schematic diagram showing processes behind the migration of additives from food packaging into food (Nerin et al., 2016)

In order to protect consumers from being exposed to toxic chemicals, laws and regulations have been put in place to govern the concentration of chemicals which can migrate from food packaging into food. In general the concentration of migrants from food packaging material should not exceed 60 mg/kg of the food it is in contact with (EU, 2011). The rate of migration of additives from packaging to food is mainly affected by the storage conditions, additive and packaging matrix type (Gavriil *et al.*, 2018). An increase in temperature and exposure time results in an increase in the rate of migration of packaging material and additives into food. Chen *et al.* (2015) reported an increase in release of eugenol and isoeugenol from protein based films as temperatures increased from 5 ^{o}C to 60^{o}C (Chen *et al.*, 2015). In addition, the food type also plays a major role in influencing the migration of additives. This can be deduced from the findings of Maryam Adilah *et al.* (2018), they reported that protein films with 5% mango kernel extract released up to 68.97 µg GAE into the aqueous simulant and 16.87 µg GAE into the fatty simulant (Maryam Adilah *et al.*, 2018). Therefore, in order to adhere with the set regulations, antioxidant release by packaging material has to be determined prior to using the material as food packaging. It is worth noting that simulants are used instead of the actual food during the testing of antioxidant release by packaging material with time as recommended by the Food and Drug Administration of the United States. The most widely used food simulants are 95% ethanol for fatty foods, 10% ethanol for alcoholic foods, deionised water for aqueous food and 3% acetic acid for acidic foods (López De Dicastillo *et al.*, 2011; García Ibarra *et al.*, 2019; Stärker and Welle, 2019).

Different rates of release of active compounds have been reported for different packaging material (Duan *et al.*, 2013; Adilah *et al.*, 2018). This has been attributed to the polarity of both the migrant and food media, interaction of the film and active compounds, film thickness and solubility of film in food simulants (Fortunati *et al.*, 2012) The use of mango extracts as an additive to packaging material to enhance food shelf life is fast growing. Antioxidants have been recovered from the leaves, kernel and peels of mango fruit and integrated into protein films, polyethylene terephthalate (PET)/polypropylene, and starch films (Maryam Adilah *et al.*, 2018; Torres-León *et al.*, 2018). However, the integration of these polyphenols into hemicellulose-based films to form active packaging with antioxidant release, is still lacking. Therefore, this study evaluated the application of hemicellulose-based films as a packaging material with antioxidant release.

Experimental evaluation of the migration of additives from packaging into food is a time consuming process and at times it is difficult to detect the migration of very low concentrations of packaging additives into food (Poças *et al.*, 2008). Therefore, models and software programs have been designed and tested on different types of films to determine their applicability in predicting migration of chemicals from food packaging. Some of the models that have been applied to estimate the migration of additives from packaging material include finite element models, Piringer models and the Limme and Hollifield models. Finite element modelling assumes that the packaging is finite and composed of small units known as mesh. The model assumes that food is in contact with the whole packaging material and that there are no voids. However, finite models do not take into consideration the effects of packaging material properties such as swelling on the migration of additives into food (Roduit *et al.*, 2005). Among the above mentioned models, the Piringer models are the simplest and have been used for modelling the migration of a wide variety of additives in packaging material. The Piringer model takes into consideration the molecular weight of the migrants, temperature at which the packaging material is exposed and the diffusion coefficients of the migrants. On the other hand, Limm and Hollifield models are limited to predicting the migration of polyolefins only from packaging. In addition, Limm and Hollifield models do not consider the geometry of the packaging and the partitioning coefficient of the packaging material which are very important aspects for food packaging migration modelling. However, the Limm and Hollifield models require easily attainable data such as activation energy and molecular mass of materials when compared to the other models (Gavriil *et al.*, 2018).

In order to overcome the limitations associated with utilising each of the above-mentioned models, software programs were developed that use more than one model in estimating the migration of packaging material components into food. Commercially available software programs, that is the SMEWISE (Simulation of Migration Experiments with Swelling Effect), Migratest and

AKTS (Advanced Kinetics and Technology Solutions) were developed based on Fick's differential equations and the finite element method (Zülch and Piringer, 2010; Bott *et al.*, 2014). These software programs have been able to estimate the release additives from food packaging made from polypropylene, paper and board (Maria Calheiros Alves Ribeiro Silva, 2009; Zülch and Piringer, 2010). Estimation of polyphenol release from hemicellulose-based films has not been studied, either experimentally and theoretically. Therefore, there is a need to evaluate the applicability of theoretical models in predicting antioxidant release from hemicellulose-based films.

2.4. Research questions

The research questions are as follows:

1. How does sequential ethanol-based organosolv extraction affect yield and quality of anthocyanins, polyphenols, and pectin from mango peels when compared to single step extraction processes?
2. What is the effect of wheat straw pre-treatment on hemicellulose modification by acetylation and the application of hemicellulose-based films as food packaging material?
3. How does increasing the DS of reinforcing nanocellulose in AH/ACNC films affect the films' hydrophobicity, solubility in food simulants and mechanical properties?
4. How do the different combinations of hemicellulose, nanocellulose and pectin affect the mechanical and functional properties of film?
5. What are the effects of temperature and time changes on the rate of antioxidant release by hemicellulose-based films into food simulants?
6. How does the antioxidant release profile of the hemicellulose-based films compare to that of LDPE films?
7. What are the effects of temperature and time on the temperature-time indicator developed from mango peel anthocyanins?

2.5. Motivation

The food packaging industry is under pressure to develop sustainable, renewable, and biodegradable packaging material similar to or better than petroleum-based packaging. Hemicellulose is among the promising alternative raw materials for developing food packaging material. The major challenges in utilising hemicellulose-based food packaging material as replacement for petroleum-based films are the films' hydrophilicity, poor mechanical properties, and high solubility in packaged food. Research has shown that the biopolymer extraction methods can influence the biopolymer composition and consequently its end-application (Li and Pan, 2018). The manipulation of biomass pre-treatment processes prior to hemicellulose extraction with the aim of developing hemicellulose-based films with enhanced mechanical properties and hydrophobicity

has not been done. The focus of research has been on the optimising of a biomass pre-treatment method for improved enzymatic digestion of biomass. Therefore, there is potential to enhance and tailor making food packaging material properties during biomass fractionation.

Preliminary research has highlighted that acetylation of arabinoxylans (DS-1.8) and reinforcing with ACNC (DS-0.54) improved the hemicellulose-based films' mechanical properties and hydrophobicity (Gordobil *et al.*, 2014). However, the effect of reinforcing AH films with ACNC having a DS beyond 0.54 has not been studied. Based on the fact that addition of a hydrophobic polymer to packaging material further enhances the hydrophobicity of material, ACNC with a DS higher than 0.54 may improve the hydrophobicity of AH/ACNC films. This could be a move towards further enhancing the properties of AH/ACNC films increasing their potential application in the food packaging industry. In addition, blending biopolymers can be a strategy towards achieving a biocomposite with desired characteristics for food packaging (Ying *et al.*, 2018; Mendes *et al.*, 2019). Biocomposites consisting of hemicellulose, pectin, and nanocellulose have not yet been evaluated as food packaging material.

More importantly, the food packaging industry is moving towards developing active food packaging. Furthermore, there is a growing need to substitute artificial antioxidants (butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT)) with natural plant antioxidants in food packaging as artificial antioxidants are feared to be carcinogenic (Quideau *et al.*, 2011). Agricultural residues can be one stop feedstocks for raw materials required in developing active packaging material and temperature-time indicators. Biorefinery processes for effective extraction of antioxidants and biopolymers can ensure the availability of functional additives to food packaging and also serve as economic methods for utilisation of agricultural residues (Arora *et al.*, 2018; Cristóbal *et al.*, 2018). The recovery processes for antioxidant and biopolymers influence the utilisation of these products as additives to food packaging. With this in mind, a novel sequential extraction process for anthocyanins, polyphenols, and pectin from mango peels was developed. Polyphenols and pectin were evaluated as additives to food packaging material. Anthocyanins' potential utilisation as temperature-time indicators was also assessed.

Furthermore, the interaction of packaging material and antioxidants plays a crucial role in determining the extent of release of antioxidants into food (Prasad and Kochhar, 2014b; Yousefi *et al.*, 2019) There is no research in which hemicellulose-based films, in particular, AH/ACNC based films and hemicellulose/pectin/nanocellulose films, have been evaluated as active packaging material with controlled release of antioxidants into food. Therefore, this project investigated both experimentally and theoretically the effect of temperature and time on the release of mango peel polyphenols from hemicellulose-based films.

2.6. Aim

The main aim of this study is to develop processes for fractionating biopolymers and bioactive compounds from wheat straw and mango waste, respectively, and their integration into a bio-composite comparable to LDPE for active food packaging.

2.7. Objectives

- To determine the effects of sequential ethanol based organosolv extraction on yield and quality of anthocyanins, polyphenols, and pectin from mango waste.
- To determine the effects of wheat straw pre-treatment before hemicellulose extraction on acetylation, hydrophobicity, and mechanical properties of hemicellulose-based films.
- To investigate the effect of reinforcing ACNC DS, loading and PCL coating on AH films' mechanical properties, hydrophobicity and solubility in food simulants using a LDPE as a benchmark.
- To experimentally and theoretically (using Micromechanical models) evaluate the reinforcement efficiency of ACNC in AH based films.
- To experimentally (according to the EU No 10/2011 directive) and theoretically (Migratest modelling) evaluate the migration of active compounds from the hemicellulose-based packaging material to different food media over time using LDPE as a benchmark.
- To assess the application of mango peel anthocyanins as temperature-time indicators.

2.8. Scientific contribution of research

1. **A sequential organosolv extraction process for recovering bioactive compounds (Chapter 4)** - New information has been generated on the impact of multistage sequential extraction of anthocyanins, polyphenols, and pectin from mango peels on the functionality of the products. The optimum conditions for successfully extracting these products from the same biomass in a biorefinery set up have been unveiled, despite the products having different optimum extraction conditions. Optimising the multiple product recovery from a single biomass is important as it enables maximum utilisation of biomass, in this case mango peels, without compromising on product functionality.
2. **A hemicellulose/nanocellulose biocomposite with mechanical properties, and hydrophobicity comparable to LDPE (Chapters 5 and 6)** -There is no literature report which assesses the effects of reinforcing AH films with ACNC having a DS higher than 0.54 and coating the films with PCL, on the films' mechanical properties, hydrophobicity and solubility in food simulants. Information generated in these chapters is essential in evaluating the potential utilisation of AH films as food packaging material.

3. **Development of hemicellulose-based active packaging (Chapter 6) -** Novel biocomposites that are AH/ACNC/PCL films and hemicellulose/pectin/nanocellulose films with controlled release of antioxidants into food simulants, were developed.
4. **Modelling antioxidant release by hemicellulose-based films into food (Chapter 6) –** The potential application of theoretical models to predict the release of antioxidants from hemicellulose-based films was presented. Mathematical models that can be used to describe the release of antioxidants by hemicellulose-based films were developed.
5. **Utilisation of mango anthocyanins as temperature-time indicators (Chapter 7) -** The response of mango anthocyanins to changes in temperature over time, was investigated. Mango anthocyanins were capable of changing colour in response to temperature and time- a property that can be exploited in the food industry to show when food has been exposed to temperatures not suitable for food storage.

3. RESEARCH DESIGN AND METHODOLOGY

This section gives an overall framework of the tasks carried out to achieve the objectives of the study. Process flowsheets for the fractionation of wheat straw and mango peels to recover biopolymers and bioactive compounds and their integration to formulate active food packaging films are presented in this section. In addition, methods used for designing experiments and analysing results are highlighted. A detailed description of experimental methods used in the study are in the relevant chapters.

3.1. Mango peel fractionation

A sequential ethanol-based organosolv extraction process was used to recover anthocyanins, polyphenols, and pectin from mango peels using conditions highlighted in Figure 13. The first stage of the sequential extraction process involved optimising the recovery of anthocyanins, based on a Box-Behnken design, taking into consideration the properties of polyphenols and pectin remaining in the residue. The anthocyanin properties considered were anthocyanin yield and antioxidant activity. The properties of polyphenols remaining in the residues evaluated were polyphenol content and antioxidant activity and for pectin were yield, antioxidant activity, UAC and neutral sugars content.

The second step involved optimising polyphenol extraction from the residues remaining after the anthocyanin extraction process. The polyphenol extraction step was performed based on a full factorial design and optimised, taking into consideration the properties of the polyphenol extract and the pectin remaining in the residue. Pectin was then extracted from the residues remaining after polyphenol extraction. The novel multiple stage mango peel fractionation process developed in this study serves to effectively extract anthocyanins, polyphenols, and pectin. This section has generated new information on how each extraction stage in a sequential mango peel fractionation process affects subsequent stages. In addition, optimum extraction conditions for the effective fractionation of the mango peels for the aforementioned biopolymers and bioactive compounds were obtained.
More information on the process of recovering anthocyanins, polyphenols, and pectin is given in chapter 4.

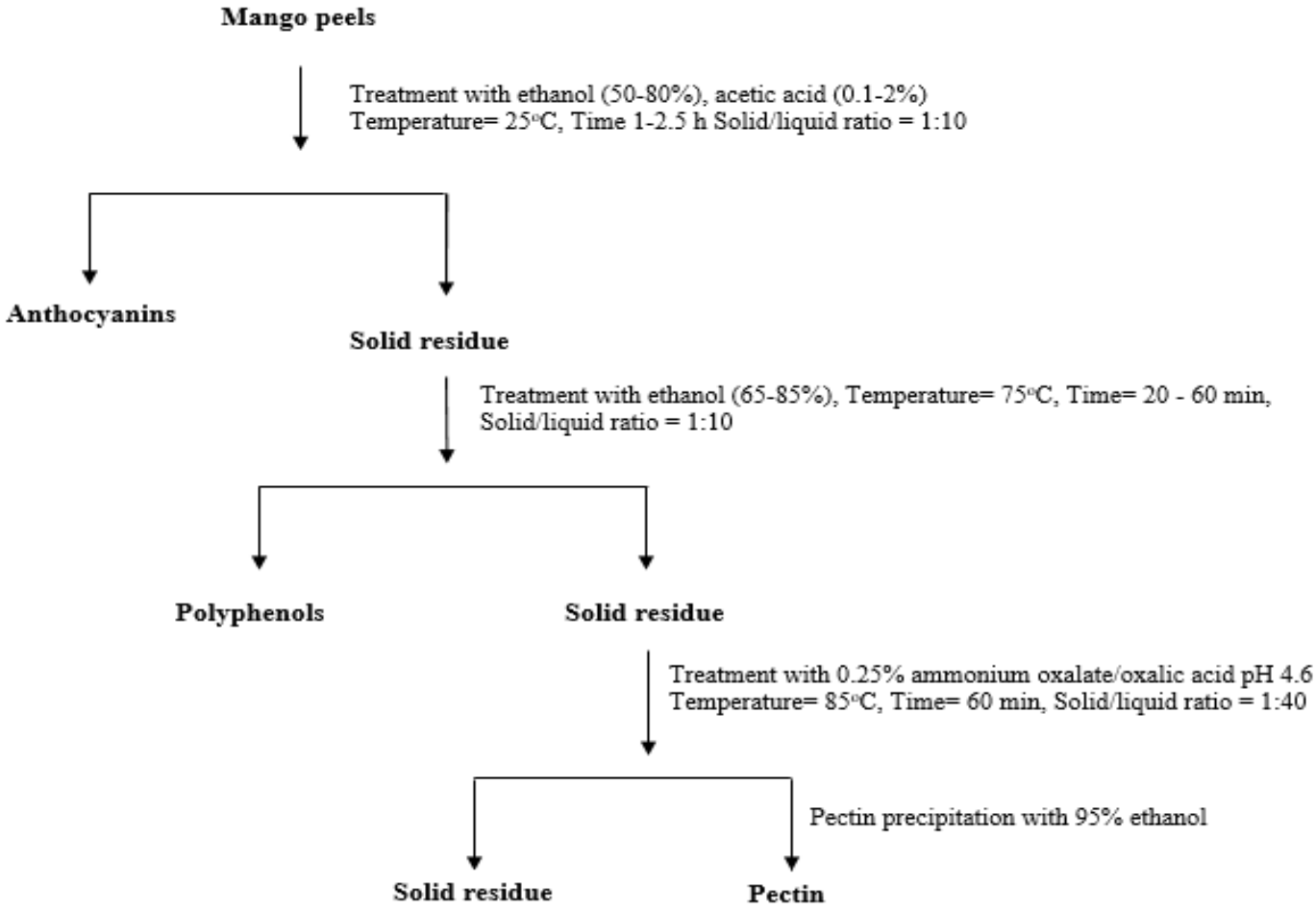

Figure 13: Process flowsheet for sequential extraction of bioactive compounds from mango peels

3.2. Wheat straw fractionation

The hemicellulose used in formulating the food packaging films was extracted from wheat straw. Wheat straw was pre-treated based on an alkali-based organosolv process prior to hemicellulose extraction using sodium hydroxide (Figure 14). A Box-Behnken design was applied to determine the effects of alkali-based organosolv pre-treatment on hemicellulose hydrophobicity, mechanical properties and acetylation. Hemicellulose was then acetylated based on the conditions highlighted in the process flow, in Figure 14. This study tailor-made hemicellulose composition before its extraction with the aim of increasing its potential utilisation as a food packaging material. Full details of hemicellulose recovery are given in Chapter 5.

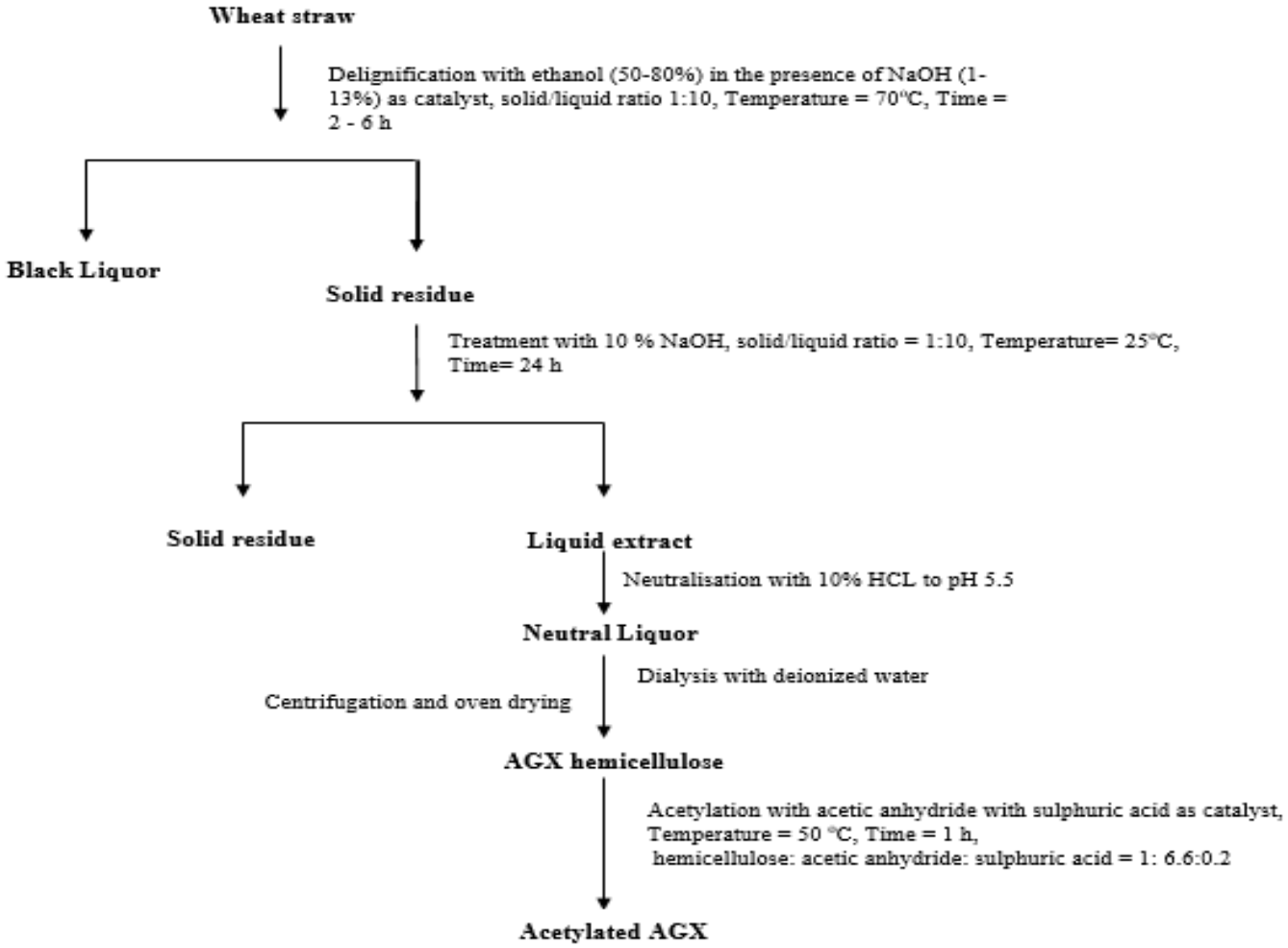

Figure 14: Process flow sheet for hemicellulose extraction and modification

3.3. Development of hemicellulose based active food packaging

Hemicellulose-based active packaging films were developed by incorporating mango peel polyphenols in the films. The solvent casting method was selected over the extrusion method for the development of hemicellulose-based active packaging, to preserve the temperature sensitive mango polyphenols that were integrated into the films. The solvent casting method can be done at temperatures as low as 25 ^{o}C, thereby minimising degradation of the polyphenols which normally degrade at temperatures higher than 70 ^{o}C (Amin, Gangurde and Alai, 2015). Hot melt extrusion requires temperatures as high as 90 ^{o}C to melt hemicellulose, hence this will lead to significant degradation of the polyphenols during the development of the hemicellulose-based active packaging films (Naczk and Shahidi, 2006; Erdemir, 2015). In addition, because of the rapid cooling of films during the solvent casting process when compared to the hot melt extrusion, the films produced by solvent casting are more uniform than those developed by extrusion. The film thicknesses of solvent casted films have smaller standard deviation values when compared to extruded films (Maniruzzaman et al., 2012). A detailed description of the development of hemicellulose-based films using solvent casting methods is given in Chapter 6.

4. DEVELOPMENT OF A SEQUENTIAL FRACTIONATION PROCESS FOR RECOVERY OF ANTHOCYANINS, POLYPHENOLS, AND PECTIN FROM MANGO PEELS.

Titles of papers contributing to the chapter

1. Box-Behnken design based multi-objective optimisation of sequential extraction of anthocyanins and pectin from mango peels
2. Mango peels biorefinery: An investigation into sequential fractionation of mango peels for anthocyanins, polyphenols, and pectin

Dissertation objectives fulfilled in this chapter

This chapter focused on developing a novel sequential multistage biorefinery route for extraction of anthocyanins, polyphenols, and pectin from mango peels (Objective 1). The three-stage extraction sequential fractionation process has the potential to preserve and enhance the functional properties (yield, antioxidant activity, purity) of the recovered products as well as increase the number of products that can be recovered from mango peels. The sequence and multi-objective recovery of the above-mentioned products from mango peels have never been reported before. The effects of each extraction stage on the properties of the products recovered and subsequent stages were investigated. Optimum conditions for the three stage extraction process were determined.

Summary of findings

The extraction stages in the multistage sequential extraction process had a significant effect on the properties of the recovered products in each stage as well as products recovered in downstream stages. The multistage extraction process was capable of extracting anthocyanins, polyphenols, and pectin with better functional properties than that recovered using single stage extraction processes, despite the products having different optimal extraction conditions. For example, pectin purity was enhanced with the sequential extraction process when compared to a single stage extraction process. In addition, instead of recovering a single product from the mango peels and discarding the residues, the multiple product extraction process enables the extraction of valuable products which could have otherwise gone to waste.

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Summary of authors' contributions

Lindleen Mugwagwa designed the experiments, conducted the experimental work and did analysis and interpretation of the results. In addition, Lindleen Mugwagwa wrote the chapter, contributing 80% of the chapter. Annie Chimphango assisted with interpretation of the results and reviewing the papers in the chapter, contributing 20% to the chapter.

4.1. Paper Title: "Box-Behnken design based multi-objective optimisation of sequential extraction of anthocyanins and pectin from mango peels".

Published in the journal of Carbohydrate polymers (impact factor 7.182)

4.2. Abstract

Multi-objective sequential fractionation of mango (*Mangifera indica* L.) peels for pectin and anthocyanins in a biorefinery, was investigated with the aims of enhancing pectin purity, bioactivity and increasing the number of recovered products. In addition, the study focused on optimising the yield and antioxidant activity of anthocyanins extracted from mango peels. Anthocyanins were extracted from mango peels based on a Box-Behnken experimental design (EC (50–80%), acetic acid concentration (AAC) (0.1–2%), time (60–150 min) and temperature (25 ^{o}C)). The remaining residue was subsequently fractionated to recover pectin. The optimum anthocyanin conditions (67.45% ethanol, 0.24% acetic acid and 60 min) resulted in pectin with a 5.5% higher antioxidant activity, it was 23% richer in polygalacturonic acid (PGA) and had a yield of 31% w/w higher than conventionally extracted pectin. An anthocyanin extract with anthocyanin content, polyphenol content, and antioxidant activity of 22.33 mg/100g, 132.62 mg/g, and 1.36 µg GAE, respectively, was obtained. Therefore, the sequential extraction of anthocyanins and pectin improves the functional properties of pectin and increases the value potential of mango peels.

Keywords: Pectin recovery; Anthocyanin recovery; Mango peels; Sequential extraction; Biorefinery; Box-Behnken design

4.3. Introduction

Mango (*Mangifera indica* L.) peels generated during the making of jams, dried chips, beverages and chutneys, are among the most underutilised fruit processing residues (Geerkens, Nagel, *et al.*, 2015). Currently, the waste is disposed of by landfilling or incineration, which results in additional costs (Deng *et al.*, 2012) because of the peels' high water content (Banerjee, Vijayaraghavan, Arora, MacFarlane, & Patti, 2016). The mango peels (40–60% of total waste) if fractionated in a biorefinery, could provide biopolymers and bioactive compounds with high economic value (Banerjee *et al.*, 2018). For example, polyphenols (93–96 mg/g), carotenoids (0.37–3.09 g/g), plant-based enzymes (amylase, protease xylanase, peroxidase and polyphenol oxidase) with activities of up to 1173 U/g (Ajila, Bhat, *et al.*, 2007; Banerjee *et al.*, 2018) can be recovered. In addition, biopolymers such as hemicellulose (8.1%), cellulose (9.19%), lignin (4.25%), and pectin (10–21.2%) can be obtained from the mango peels (Orozco *et al.*, 2014; Kermani *et al.*, 2015). However, pectin and polyphenols are the most economically promising products due to their abundance and ease of extraction from the mango peels (Banerjee *et al.*, 2018).

Pectin is a polysaccharide with linear linkages of D-galacturonic acid (GA) containing either free amidated or methyl esterified carboxyl groups bonded together by α-1,4-glucosidic bonds (Raj *et al.*, 2012). The pectin chains consist of 300 to 1000 units of galacturonic acid. Pectin is classified into two groups based on the degree of esterification of the galacturonic acids. That is pectin with a degree of esterification higher than 50% are classified as high methoxyl pectin and that which has less than 50% are called low methoxyl pectin. Depending on pectin source, pectin consist of side chains of neutral and acidic sugars. The current sources of high-quality pectin (having GA content > 65%) include apple pomace, citrus peels, sugar beet and sunflower (May, 1990; Sila *et al.*, 2009). Apart from the pectin source, the pectin extraction conditions (solvent type, temperature, pH), contribute significantly to pectin functional properties, thereby affecting its end application (Dorta *et al.*, 2012; Pasandide *et al.*, 2017).

Pectin is extracted by acid–assisted methods followed by precipitation with alcoholic solvents and metallic salts (Pasandide *et al.*, 2017). The conventional acid–based extraction (CEP) produces brown coloured pectin (rich in polyphenols and neutral sugars), which limits its application, particularly, in food packaging and as drug release agents (Sila *et al.*, 2009; Srivastava and Malviya, 2011). The migration of polyphenols from packaging (allowable limit is < 10 mg/dm^2 of the packaging contact area) into food is a major food safety concern, limiting the utilisation of polyphenol-rich packaging materials. (EU, 2011; López de Dicastillo *et al.*, 2016). Furthermore, a combination of high neutral sugar and low GA content (56–72.5%) in pectin recovered from the CEP, promotes gelling and coagulation affecting the uniformity of the packaging films (Raj *et al.*,

2012). Pectin with high purity (pectin with high galacturonic acid content and minimal side branches) and functionality can be achieved by adopting extraction conditions, which favour high GA content and antioxidant activity, and minimal neutral sugar and phenolic contents (Chaharbaghi *et al.*, 2017).

The red coloured mangoes (Kent, Tommy Atkins, Haden and Keitt) are the preferred cultivars in mango processing industries because they are less fibrous and can be transported over long distances (Caballero, Finglas & Toldra, 2015). The peels of these cultivars can be exploited as new sources of anthocyanins (565 mg/100 g, mainly cyanidin 3-*O*-glucoside and peonidin 3-*O*-glucoside). Anthocyanins are utilised as bioactive colourants (Berardini, Fezer, *et al.*, 2005; Miguel, 2011; Ravani and Joshi, 2013), and the current sources are grape pomace, potatoes, black currants, carrots, and red cabbage leaves (Miguel, 2011). Extraction conditions that result in high yields and antioxidant activity of anthocyanin extracts are desirable for recovering anthocyanins for utilisation as natural colourants (Nour *et al.*, 2013; Chockchaisawasdee and Stathopoulos, 2017).

Anthocyanins and pectin are traditionally extracted in stand-alone processes. In pectin extraction, the anthocyanins are degraded prior to pectin extraction whereas, in anthocyanin extraction, the alcohol-insoluble residue (AIR) is discarded after anthocyanin extraction (Nour *et al.*, 2013; Joshi and Preema Devi, 2014; Banerjee *et al.*, 2016). Simultaneous extraction of the two products has significant limitations due to the differences in optimal extraction conditions, especially temperature and time (pectin; 85 ^{o}C, 1 h and anthocyanins; 25 ^{o}C, and up to 24 h) (Cacace & Mazza, 2003; Koubala et al., 2008). Co-extraction under conditions favourable for pectin will degrade anthocyanins. On the other hand, the low temperatures at which anthocyanins are extracted cannot effectively extract the pectin from the mango peels. Notably, the temperature (> 70 ^{o}C) at which the alcohol-soluble material is removed from mango peels before pectin extraction degenerates the anthocyanins (do Nascimento Oliveira *et al.*, 2018). Therefore, multi-objective optimisation of a sequential extraction process (SEP) in which both pectin and anthocyanins are to be extracted from the same feedstock, is necessary to maintain their functional properties and as a step towards exploiting the full economic potential value of the waste.

Many researchers have applied an alcoholic pre-treatment step to remove the alcohol-soluble material before extracting the pectin (Kermani et al., 2014; Koubala et al., 2008). Understanding the effects of varying mango peel pre-treatment conditions on the pectin composition can provide strategies for improving pectin purity. However, most studies on pectin extraction have been on the effects of the CEP conditions on pectin recovery from the AIR (do Nascimento Oliveira et al., 2018; Kermani et al., 2014; Koubala et al., 2008), thus, without considering the effects of the alcohol pre-treatment on the functional properties of the pectin from the AIR. Moreover, the effect of

anthocyanin pre-extraction from mango peels prior to pectin extraction on pectin properties is unknown. Anthocyanin composition per se is among other factors, influenced by the extraction conditions (Nour *et al.*, 2013). Yet, research mainly focuses on the identification and quantification of the mango peel anthocyanins and not on the optimisation of the extraction conditions (Berardini, Fezer, *et al.*, 2005; Ajila, Bhat, *et al.*, 2007). Optimisation of the sequential extraction of anthocyanins and pectin from mango peels has the potential to increase the recovery of functional products from mango peels. Thus, multi-objective optimisation of the extraction conditions for effective recovery of both the anthocyanins and pectin from the mango peels is essential. This study aimed at developing a SEP for pectin and anthocyanins from Kent mango peels. The effects of the SEP conditions on the properties of pectin (yield, neutral sugars and polyphenol content, UAC and degree of esterification (DE)) and the anthocyanins (anthocyanin content, polyphenol content, and antioxidant activity) were investigated based on a Box-Behnken experimental design. A multi-objective statistical optimisation technique called the Derringer's desired function methodology (Boudaoud *et al.*, 2006) was applied to identify optimal conditions for selective extraction of the two products.

4.4. Materials and methods

4.4.1. Samples preparation

Fully ripened Kent mangoes (*Mangifera indica* L.) purchased from the Cape Town market, South Africa, were peeled, and the peels sun-dried at 40 ^{o}C in a greenhouse for one week. The peels were milled with a laboratory retch mill (Retch ZM200 mill with a 2 mm circular blade) to a particle size < 800 µm. The peels were stored in tightly closed glass bottles wrapped with aluminium foil before experimental work. Folin Ciocalteu, DPPH, acetic acid, gallic acid, pectin standard, HCl, carbazole, GA, sugar standards (xylose, glucose and arabinose) and sulphuric acid were purchased from Sigma Aldrich (Johannesburg, South Africa) and ethanol, sodium acetate, NaCl, sodium carbonate, ammonium oxalate, oxalic acid, NaOH, KOH and phenolphthalein were sourced from Science World (Cape Town, South Africa). All chemicals were of analytical grade.

4.4.2. Ethanol-based extraction of anthocyanins

Mango peels (25 g) were homogenised in ethanol/acetic acid solutions (250 mL) consisting of a 50–80% ethanol and 0.1–2% AAC giving a solid /liquid ratio of 1:10 (25g: 250 mL) with a high-speed blender (220–240 V, 50 Hz, 250 W) for 1 min. The extraction was carried out at 25 ^{o}C for 1–2.5 h while stirring with a magnetic stirrer at 800 rpm. The hydrolysate was centrifuged for 20 min at 8000 rpm. The recovered supernatant (230 mL) was kept at -4 ^{o}C in plastic tubes wrapped with aluminium foil before further analysis. The solid residue was washed 3 times with water (500 mL), oven dried at 40 ^{o}C (moisture content < 10%) for 72 h, and stored in sealed bags.

4.4.3. Determination of anthocyanin extract's anthocyanin content, polyphenol content, and antioxidant activity

4.4.3.1. Total anthocyanin content determination

The total anthocyanin content was determined according to the method by Giusti & Wrolstad (2001). The anthocyanin extract (3 mL) was added to 10 mL of NaCl buffer at pH 1 and to sodium acetate buffer at pH 4.5. The solutions were left standing in the dark for 30 min at 25 ^{o}C. The absorbance was measured at $A_{\lambda\ \text{vis-max}}$ = 510 nm and λ = 700 nm using a Varian Cary 50 Bio UV-Visible (UV-VIS) spectrophotometer. The absorbance of the extracts (A) was calculated using Equation 1.

$$A = (A_{\lambda\ vis-max} - A_{700})_{pH\ 1} - (A_{\lambda\ vis-max} - A_{700})_{pH\ 4.5} \quad (1)$$

where:

A = Extract absorbance

$A_{\lambda\ \text{vis-max}}$ = Extract absorbance at 510 nm,

A_{700} = Extract absorbance at 700 nm.

The anthocyanins concentration was calculated using Equation 2.

$$Anthocyanin\ content\ (mg/g) = (A \times MW \times DF \times 1000)/(\varepsilon \times \lambda \times m) \quad (2)$$

where:

MW = Anthocyanin molecular weight;

DF = Dilution factor,

ε = Molar absorptivity,

m = Mass of peels

λ = Lightpath length

The anthocyanins were quantified as the sum of the concentrations of cyanidin 3-O-glucoside and the peonidin 3-O-glucoside in the anthocyanin extract.

4.4.3.2. Total polyphenols content analysis

The total polyphenol content was determined according to a method by Ribeiro, Barbosa, Queiroz, Knödler, & Schieber (2008). The anthocyanin extract (0.5 mL) was mixed with Folin-Ciocalteu reagent (0.5 mL) followed by 7.5% sodium carbonate (0.5 mL). The mixture were left for 30 min before measuring absorbance at 765 nm using a Varian Cary 50 Bio UV-VIS spectrophotometer. A blank sample containing all the reagents except the mango peels extract was used as a reference. The polyphenol content of the extracts was expressed as μg of GAE.

4.4.3.3. Antioxidant activity analysis

The antioxidant activity of the anthocyanin extracts was analysed by a procedure adapted from Ribeiro et al. (2008). Mango peel extract (100 µL) (with polyphenol concentrations of 1000 - 5000 ppm) was mixed with 0.1 mM DPPH (5 mL) in 80% ethanol. The mixtures were left in the dark for 30 min at 25 ^{o}C. The absorbance for both the mixtures and the control was measured at 517 nm using a Varian Cary 50 Bio UV-VIS spectrophotometer. The DPPH scavenging activity was calculated using Equation 3.

$$DPPH\ Scavenging\ Activity = \left(\frac{A_1 - A_2}{A_1}\right) \times 100 \quad (3)$$

where:

A_1 = DPPH absorbance

A_2 = DPPH absorbance of the anthocyanin-DPPH mixture.

The antioxidant activity was recorded as IC_{50}, which is a measure of the anthocyanins concentration that can scavenge 50% of DPPH. The DPPH scavenging activities were plotted against polyphenol concentrations from which the IC_{50} values were estimated. The higher the IC_{50} value the lower the antioxidant activity of the extract and vice versa (Ajila, Bhat, *et al.*, 2007).

4.4.4. Ammonium oxalate based pectin extraction and quantification

Pectin was extracted from both mango peels and the residue after anthocyanin extraction, using the method by Koubala *et al.* (2008) on a reduced AIR sample size, 5 g instead of 100 g. The mango residue (10 g) was treated four times with 85% ethanol (30 mL) at 70 ^{o}C, each cycle lasting 20 min, to remove alcohol-soluble material. The AIR (8.117–8.633 g) was washed 3 times with water (500 mL), and then oven dried at 40 ^{o}C to a moisture content < 10%. Pectin was extracted from 5 g AIR with 0.25% ammonium oxalate/oxalic acid (200 mL), resulting in a solid /liquid ratio of 1:40, for 1 h in a water bath at 85 ^{o}C. The pectin supernatant (186 mL) was filtered followed by precipitation with 400 mL of 95% ethanol at 25 ^{o}C. The pectin was then washed three times with 70% ethanol (100 mL) followed by 90% ethanol (100 mL) washing before being oven dried at 40 ^{o}C for 24 h. As a benchmark, pectin was extracted from 5 g mango peels using 200 mL of 0.25% ammonium oxalate/oxalic acid under similar conditions as described for pectin extraction from the AIR, without prior removal of the alcohol-soluble material (the method is called the CEP in this study).

Pectin yield was calculated as follows (Equation 4):

$$Pectin\ (\%) = \frac{Weight\ (g)\ of\ dried\ pectin}{Weight\ (g)\ of\ mango\ peels\ used\ for\ extraction} \times 100\% \quad (4)$$

4.4.5. Estimating pectin degree of esterification

The pectin samples were analysed for DE with a titration method by Pasandide *et al.* (2017). A pectin sample (200 mg) was dissolved in ethanol (3 mL), and subsequently, diluted with deionised water (20 mL) before titrated with 0.1 N NaOH using phenolphthalein as an indicator. The end-point volume of NaOH was recorded as the initial titre. About 10 mL of 0.1 N NaOH was added to the mixture while mixing before leaving it for 15 minutes at 25 ^{o}C. Thereafter, 0.1 N HCl (10 mL) was added while shaking until the pink colour disappeared. Phenolphthalein indicator (five drops) was added and the solution was titrated again with 0.1 N NaOH until a faint pink colour was observed signalling the end-point. The end-point NaOH volume was recorded as the final titre. The DE was calculated using Equation 5.

$$DE\ (\%) = \frac{Final\ titer}{Initial\ titer + final\ titer} \times 100 \qquad (5)$$

where: DE = degree of esterification

4.4.6. Determination of pectin neutral sugars content and uronic acid content

Pectin was hydrolysed following the methods described by Koubala *et al*. (2008). Pectin (500 mg) was hydrolysed with 10 mL of 1 M sulphuric acid for 3 h in a shaking water bath at 100 ^{o}C. The hydrolysate pH was adjusted to a pH between pH 3 and pH 7 by addition of 7 M KOH followed by filtering (0.22 µm nylon filters) before determining neutral sugars by high-performance liquid chromatography (HPLC) on a BioRad Aminex HPX-87 column equipped with a Cation-H Micro-Guard Cartridge and Shodex R I101a refractive index detector. The mobile phase (5 mM sulphuric acid) was set at a flow rate of 0.6 mL/min at 65 ^{o}C. Sugar standards (xylose, glucose, and arabinose) calibration curves were used to quantify the respective sugars.

The pectin UAC was determined according to the methods by Li, Kisara, Danielsson, Lindström & Gellerstedt (2007) and Brienzo, Siqueira & Milagres (2009) used for the determination of UAC from hemicellulose hydrolysate. However, in this study the pectin hydrolysate was prepared according to the pectin hydrolysis method used in the neutral sugars determination method above. The pectin hydrolysate (0.4 mL), was mixed with concentrated sulphuric acid (2.4 mL) in a stoppered test tube. Subsequently, 0.1 w/v carbazole in 95% ethanol (0.1 mL) was added and the mixture heated at 100 ^{o}C in a water bath for 20 min, after which it was cooled to 25 ^{o}C in an ice-bath. The absorbance of the mixture was measured at 525 nm using a Varian Cary 50 Bio UV-VIS spectrophotometer. UAC was quantified based on a GA calibration curve.

4.4.7. Determination of pectin polyphenol content

The pectin polyphenol content was determined according to Rombouts &Thibault (1986) from a pectin sample weighing 50 mg instead of 25 mg as indicated in the original method. The pectin sample (50 mg) was hydrolysed with 0.5 M KOH (2 mL) in a water bath at 60 ^{o}C for 90 min. The hydrolysate was cooled to 25 ^{o}C in an ice bath before adjusting the pH to pH 2 with 32% HCl. Thereafter, the polyphenols were extracted from 2 mL of the hydrolysate by adding 6 mL of 95% ethanol and incubating the mixture in a shaking water bath for 1 h at 25 ^{o}C. The polyphenol content was quantified as per the method provided in Section 4.4.3.

4.4.8. Determination of pectin antioxidant activity

Pectin antioxidant activity was determined according to Jae *et al*. (2011) with modifications. A 1 mL pectin solution (1 mg/mL) was tested for free radical scavenging capabilities by adding 1 mL of 0.1 mM DPPH in ethanol instead of the 50 µM used in Jae *et al.* (2011) due to the pectin exhibiting high scavenging capabilities, which affected the accuracy of the measurements. The mixture was heated in the dark in a water bath at 60 ^{o}C for 30 min. The absorbance was measured at 517 nm using a Varian Cary 50 Bio UV-VIS spectrophotometer. The antioxidant activity was calculated using Equation 3.

4.4.9. Pectin structural characterisation using Fourier transform infrared spectroscopy (FTIR)

The structural characteristics of the extracted pectin and commercial pectin were determined by using a Nexus model FTIR spectrometer fitted with a Golden Gate diamond single reflectance ATR sampling accessory (Thermo Scientific, Nicolet, USA). The absorbance was measured from 650 to 4000 cm^{-1} at a resolution of 8 cm^{-1} with 64 co-added scans. The FTIR spectra were processed using the Origin 6 software (OriginLab cooperation, USA) and the bands were assigned according to Banerjee *et al*. (2016).

4.4.10. Box-Behnken based experimental design for optimisation of pectin and anthocyanins sequential extraction process

The effect of extraction conditions on the anthocyanins and pectin properties were assessed in a Box-Behnken experimental design in which three factors, EC (50–80%), AAC (0.1–2%), and time (60–150 minutes) were varied at three levels consisting of 15 runs with three centre points (Table 6). The two-stage extraction process was optimised based on the multi-objective Derringer's desired function methodology (Boudaoud *et al.*, 2006) in Statistica 7 software, version 13.2). All response variables were analysed in triplicate.

Table 6*: *Mango peels fractionation Box-Behnken experimental design process conditions

Run	Ethanol concentration (%)	Acetic acid concentration (%)	Extraction time (minutes)	Design levels (Coded values)		
	X_1	X_2	X_3	X_1	X_2	X_3
1	50	0.1	105	-1	-1	0
2	80	0.1	105	1	-1	0
3	50	2	105	-1	1	0
4	80	2	105	1	1	0
5	50	1.05	60	-1	0	-1
6	80	1.05	60	1	0	-1
7	50	1.05	150	-1	0	1
8	80	1.05	150	1	0	1
9	65	0.1	60	0	-1	-1
10	65	2	60	0	1	-1
11	65	0.1	150	0	-1	1
12	65	2	150	0	1	1
13	65	1.05	105	0	0	0
14	65	1.05	105	0	0	0
15	65	1.05	105	0	0	0

4.5. Results and discussions

4.5.1. Effect of pre-extraction conditions of anthocyanins on anthocyanins and pectin properties

Anthocyanin extracts with an anthocyanin content as high as 39.52 mg/100g, polyphenolic content of up to 128.9 mg/g GAE and antioxidant activity as high as 0.13 µg GAE were recovered from the mango peels (Table 7). The anthocyanin extraction stage left a residue (37.6 – 44.7% of the original mango peels) of which 81–85.6% was the AIR, available for pectin extraction (Table 7). The residue yielded good quality pectin (124.2–228.7 mg/g) with purity as high as 85.13% (based on UAC) and could scavenge up to 91.7% of DPPH free radicals (Table 7).

4.5.1.1. Effect of extraction conditions on anthocyanin yield and antioxidant activity

Anthocyanins were recovered in high yields with high antioxidant activity (Table 7). Regardless of the feedstock type, anthocyanin yield tends to increase with EC up to a concentration of approximately 60%, thereafter, a decrease in yield is observed (Cacace and Mazza, 2003; Xavier *et al.*, 2008; Nour *et al.*, 2013). However, in this study, the highest yield was obtained at above 70% ethanol, with an optimum at 80% (Figure 15a and b). Although there is no study in which the effect of EC on the anthocyanin yield from mango peels is reported, the differences between the EC for maximum anthocyanin yield for this study and the literature can be due to the differences in feedstock varieties and species. Vatai, Škerget, and Knez's (2009) work showed that the highest

anthocyanin yield for one specific grape variety (Cabernet) was attained at 100% EC but for other grape varieties (Merlot and Refok), the optimum was about 50%.

Similar to anthocyanins (Figure 15a, b and c), the polyphenol content increased with an increase in EC and AAC but was less dependent on the treatment time (Figure 15d, e and f). In contrast, the higher anthocyanin polyphenol content was obtained at ECs between 60% and 70% (Figure 15d and e) instead of the 70–80% (Figure 15a and b). This result is an indication that obtaining higher anthocyanin content would compromise polyphenol yield in the anthocyanin extract.

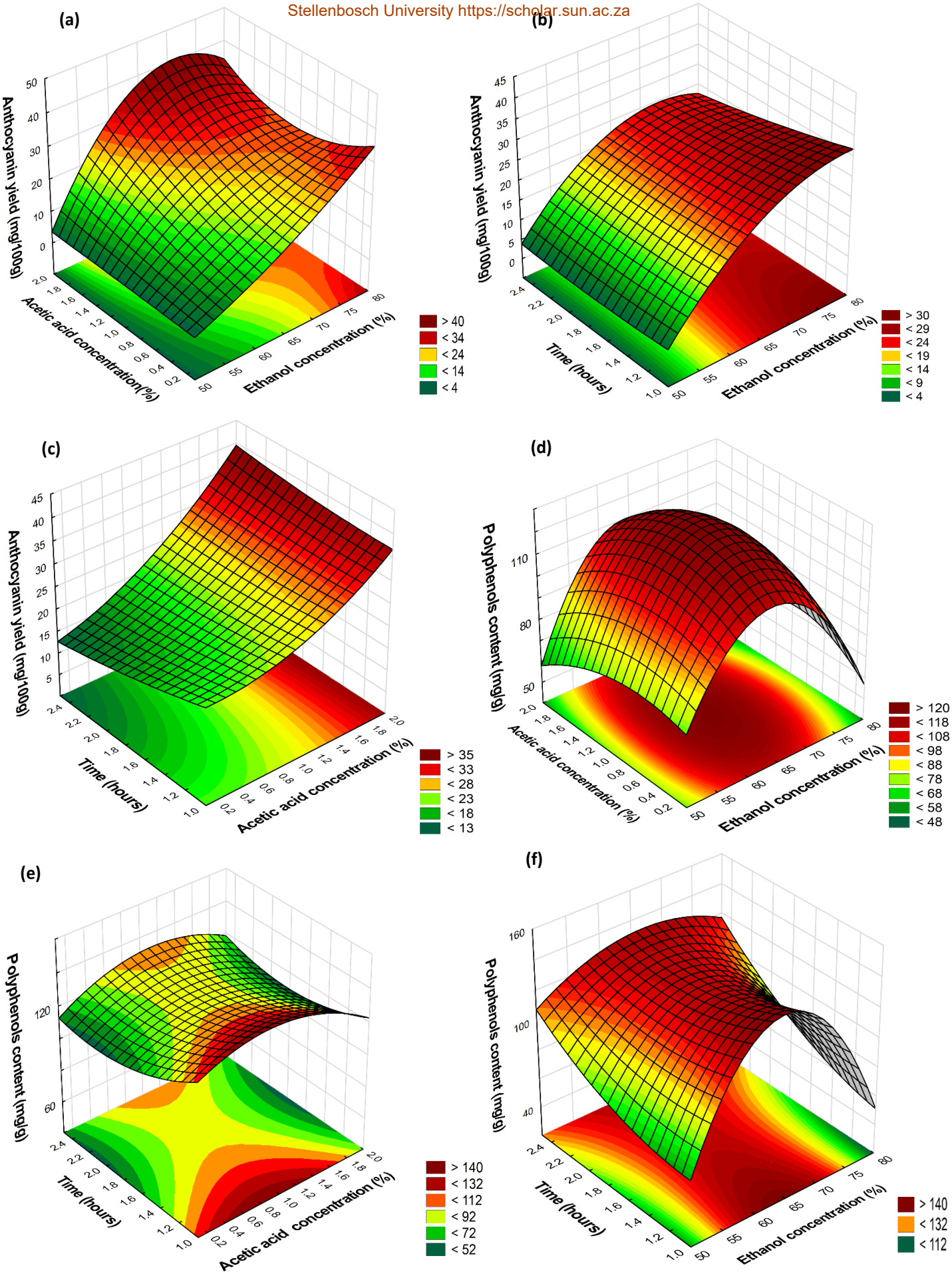

Figure 15. Response surface plots for the effects of anthocyanin extraction conditions on anthocyanin yield and polyphenol content

The increase in polyphenol yield with an increase in AAC can be attributed to reduced oxidative enzyme activity in the polyphenol extract at low pH levels. Oxidative enzymes are known to compromise the stability of the extracted polyphenols (Tunchaiyaphum *et al.*, 2013).

Anthocyanin antioxidant activity increased in the EC range from 65% to 80%, irrespective of treatment time (Figure 16 a and b). The antioxidant activity of the anthocyanin extracts is attributed to the availability of hydroxyl groups on the aromatic structure of anthocyanins and polyphenols present. These have the ability to donate hydrogen molecules that scavenge the free radicals from the DPPH causing a decolouration of DPPH. In addition, minimised co-extraction of other carbohydrates with the anthocyanin at high ECs (80%) resulting in lower interference in scavenging activities of the hydroxyl groups could have also favoured high antioxidant activity in the anthocyanin extracts. (Ajila, Naidu, et al., 2007; Ribeiro et al., 2008). The range coincided with the optimal range for high anthocyanins yield (Figure 15a and b) and a decline in polyphenols (Figure 15d and f) implying that a high anthocyanin content boosted antioxidant activity whereas a high polyphenol content does not necessarily improve antioxidant activity. Therefore, it is important to control EC during extraction in order to maximise the yield of anthocyanin extracts with high antioxidant activity.

The reduction in antioxidant activity when anthocyanins are extracted with an AAC higher than 0.4% for longer periods (Figure 16c) might lead to the co-extraction of compounds that interfere with scavenging of the free radicals. The co-extracted components, which also include reducing sugars, form glycosides with the polyphenols in the anthocyanin extract by a condensation reaction. The bonding of the polyphenols to the co-extracted components, diminish free hydrogen donors available to scavenge free radicals (Shalaby et al., 2016). Notably, understanding the effects of anthocyanin extraction conditions on the properties of the anthocyanins would enable the manipulation of these extraction parameters to enhance the targeted product properties in a SEP.

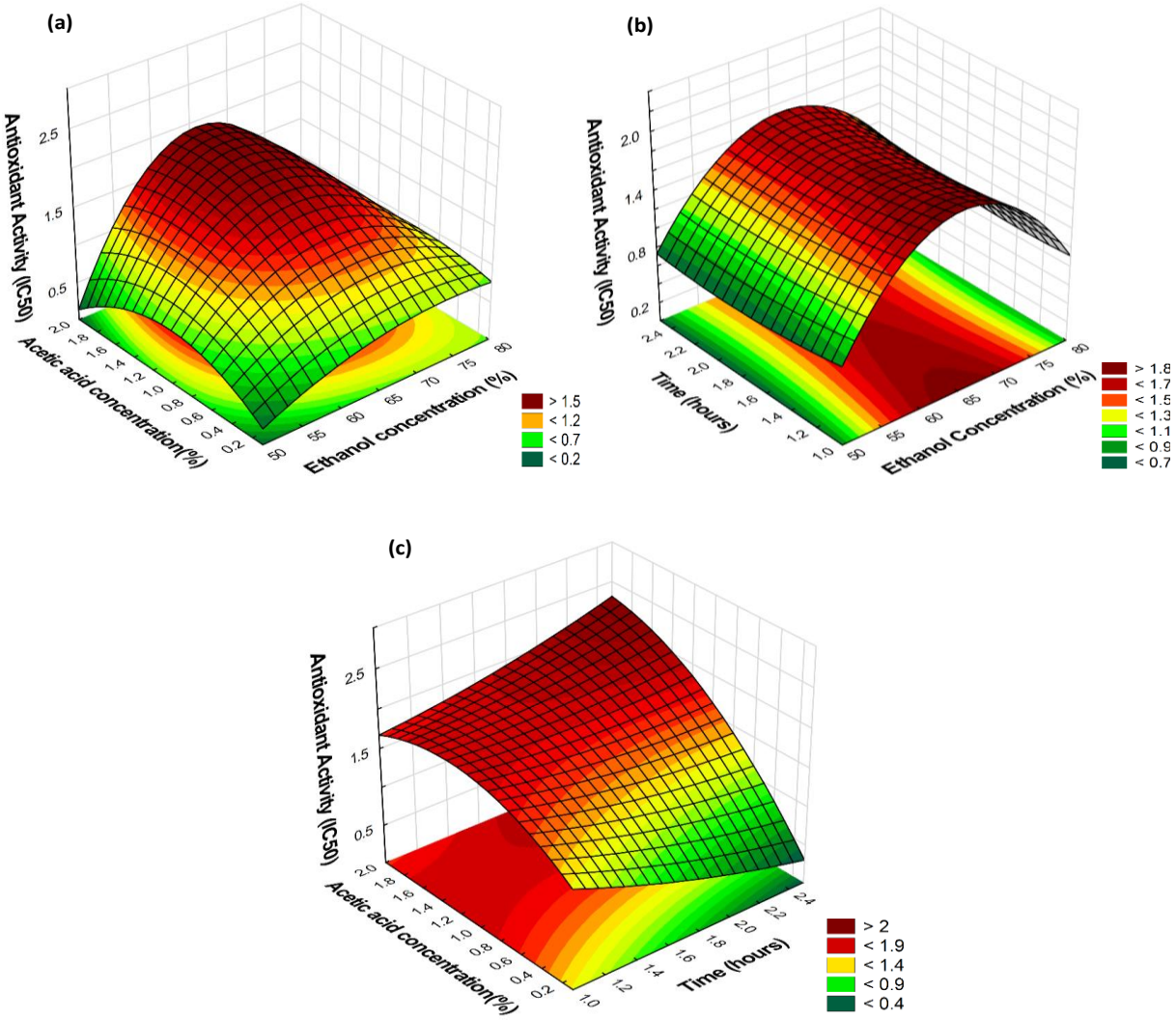

Figure 16 3-D surface response graphs of the effects of anthocyanin extraction variables on anthocyanin antioxidant activity

4.5.2. Effect of anthocyanins pre-extraction conditions on pectin yield and properties

Higher yields of pectin, on a feedstock basis, were obtained from the anthocyanin-free residue (120–230 mg/g) (Table 7) when compared to the original mango peels (163 mg/g on AIR) (Table 8). Pectin yields after the SEP, were within the results by Koubala *et al*. (2008) regardless of the pre-extraction of anthocyanins. The anthocyanins extraction conditions could have been too mild to cause a drop in yield or structural change to the pectin in the residue. More importantly, the pectin recovered after the SEP (656–851.3 mg/g) had higher UAC (Table 7) when compared to pectin obtained from the CEP (620 mg/g) (Table 8). Considering that the pectin from the SEP had a UAC above 650 mg/g (Table 7) whereas that from the CEP had a UAC less than 650 mg/g (Table 8), it can be concluded that the removal of anthocyanins before pectin extraction produces good quality pectin.

In addition, the pectin antioxidant activity was enhanced by the SEP as evidenced by the higher free radical scavenging capabilities when compared to pectin from the CEP (Table 8). Hence the extraction of anthocyanins before pectin improved both pectin purity and antioxidant activity. Therefore, these results support the utilisation of the residue after anthocyanins extraction as a source of good quality pectin. Also, the SEP improves the functionality of the products and increases the number of products recovered from mango peels.

On the contrary, the pectin yield of the SEP (39.7–73.8 mg/g) (Table 7) based on the original mango peels fractionated was lower than that of the CEP (163 mg/g) (Table 8). The higher yield in pectin for the CEP could be because of co-extraction of the PGA with phenolics and neutral sugars. The phenolics and neutral sugars in the pectin lowered the UAC, hence, lowering pectin quality. The yield range for SEP obtained in this study is similar to that of pectin obtained from mango peels that were blanched before extraction (under similar conditions (alcoholic solution at pH 2.5 and pH 3)) (Pedroza-Islas *et al.*, 1994). Despite low pectin yields (Table 7), SEP pectin has potential application as drug release agents and as food-packaging films, applications where purity is a concern (Peng and She 2014).

Table 7: Mango peel residues, anthocyanins and pectin properties at different extraction conditions.

	Anthocyanins extract			**Residues**		**Pectin**					
Run	Anthocyanins yield (mg/100g)	Polyphenol (mg/g GAE)	Antioxidant activity (µg GAE)	Pre-extracted residue (%)	AIR (%)	Yield (AIR basis) (mg/g)	Yield dry weight basis (mg/g)	Uronic acid content (mg/g)	Polyphenol content (mg/g GAE)	Neutral sugars (mg/g)	Antioxidant activity (%)
1	3.06	73.74	0.21	40.84	81.17	144.9	53.4	656.0	17.72	243.4	87.59
2	34.79	56.62	0.77	43.52	82.00	131.0	51.7	744.1	13.37	171.5	85.42
3	3.50	57.93	0.13	39.08	83.67	210.0	65.8	772.2	16.85	105.7	80.35
4	34.28	46.41	0.54	43.48	83.17	146.7	56.5	810.4	16.73	180.7	91.74
5	4.29	65.13	0.82	40.72	84.20	200.4	63.7	806.0	21.23	175.3	66.00
6	31.62	50.93	0.91	44.64	81.00	167.2	61.6	756.3	21.40	147.2	88.93
7	3.84	107.12	0.73	38.16	82.8	198.8	61.5	851.3	20.51	130.4	62.75
8	22.54	116.78	1.06	40.48	84.54	170.0	45.6	808.7	18.20	164.6	75.00
9	21.59	133.71	1.28	39.32	83.08	197.8	59.5	790.7	18.06	147.0	85.96
10	36.62	122.33	1.67	40.60	82.54	228.7	70.1	743.3	29.56	152.2	90.73
11	12.63	112.63	0.30	42.40	84.70	141.8	52.4	80.25	29.36	183.4	60.39
12	39.52	118.35	2.31	37.60	86.33	124.2	39.7	724.6	28.77	162.8	73.29
13	22.71	127.01	1.71	39.80	84.53	183.7	57.0	777.2	26.86	192.0	72.31
14	23.88	126.20	1.65	38.50	85.82	190.1	73.8	819.6	20.55	221.0	85.13
15	22.50	128.90	1.75	39.50	85.61	183.1	62.6	760.0	16.48	187.0	83.15
Factor	**P-Values (3 decimal places)**										
(1)EC	0.000	0.000	0.000			0.000		0.280			
(2)AC	0.000	0.000	0.000			0.000		0.012			
(3)T	0.000	0.000	0.012			0.000		0.070			
1*2	0.000	0.000	0.000			0.001		0.006			
1*3	0.001	0.001	0.008			0.000		0.264			
2*3	0.000	0.000	0.000			0.000		0.477			

- EC: Ethanol concentration, AC: Acetic acid concentration, T: Time, AIR: Alcohol insoluble residue, GAE: gallic acid equivalent

Table 8: Physicochemical properties of sequentially (at optimum conditions) and conventionally extracted pectin

Processes	Pectin yield (mg/g)	Uronic acid content (mg/g)	Degree of esterification (%)	Antioxidant activity (%)
CEP	163	621	70	92.21
SEP	215	766	70	97.17

CEP: conventional extraction process; SEP: sequential extraction process;

The pre-extraction of anthocyanins before pectin extraction had a significant effect on the pectin yield and UAC (p <0.05) (Table 7). Varying anthocyanins pre-extraction conditions affected the pectin composition differently with regard to UAC, polyphenol and neutral sugars contents, resulting in differences in pectin weight (Figure 17 and 18). The SEP yields of anthocyanins and pectin were inversely related (Figure 17a). The anthocyanins extraction conditions that enhanced UAC boosted pectin yields (Figure 17b) but negatively affected pectin neutral sugars and polyphenol content (Figure 17c, d and e). An increase in UAC was realised with extended exposure to AAC. The longer contact time between the acid and the peels could have accelerated hydrolysis of bonds between the GA and associated neutral sugars as well as the polyphenols (Figure 17c and d), thereby removing possible impurities (Xavier *et al.*, 2008). Thus, GAs are the major contributors to the pectin molecular weight. However, removal of the neutral sugars and polyphenols from pectin improves the purity of the pectin at the expense of its antioxidant activity (Figure 17 g and f). Furthermore, lower ECs, thus high water content in the anthocyanin extraction medium could have promoted the dissolution of water-soluble neutral sugars during the anthocyanins extraction, leaving pectin in the residue with less associated neutral sugars and polyphenols but with more GA (Figure 17c and e). Pectin yield from the anthocyanin-free residue was boosted with a reduction in the EC from 80% to 50% (Figure 18a and d). However, at the same ECs, the anthocyanins yield decreased (Figure 18a, b, d and f).

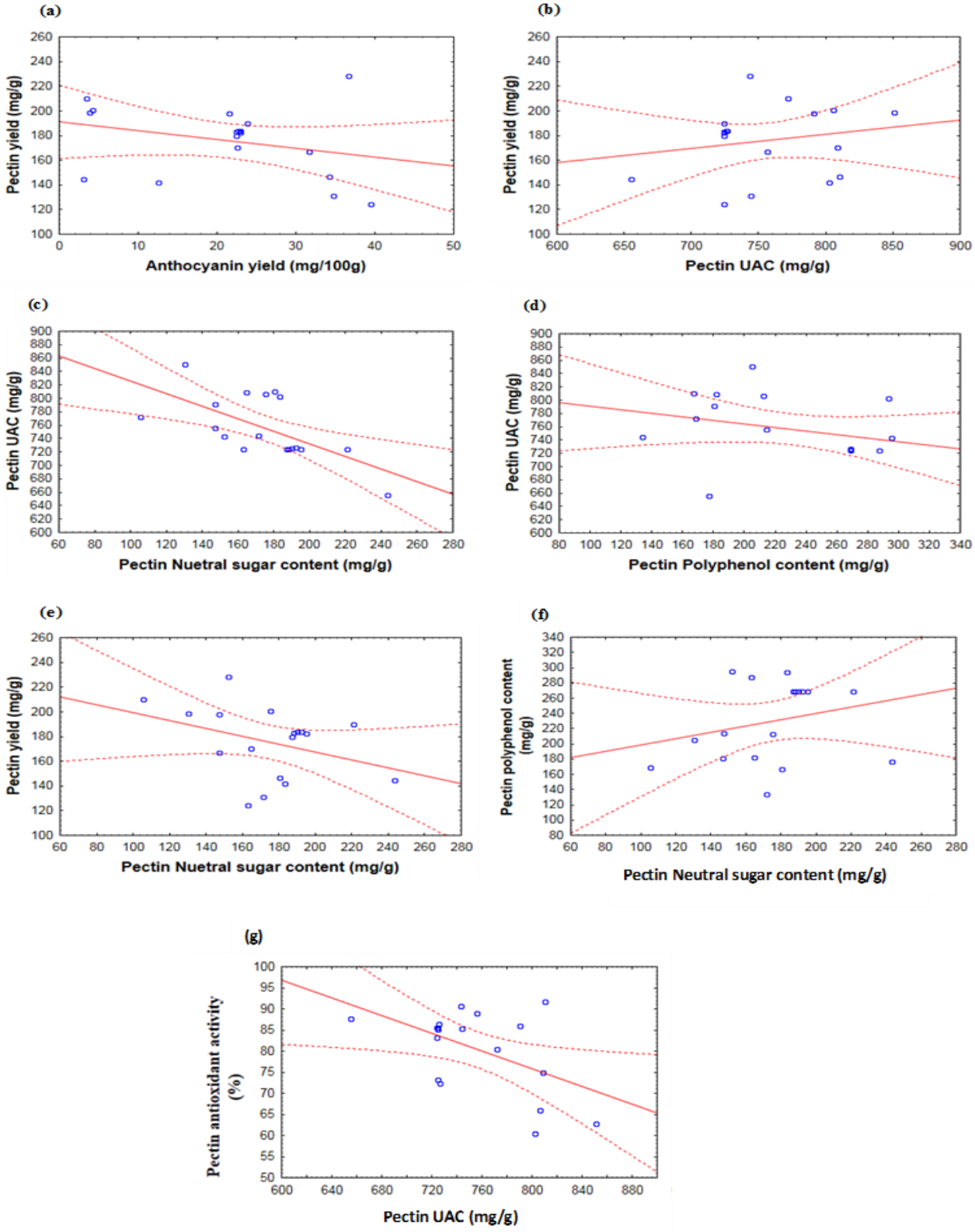

Figure 17: Correlation of pectin compositional properties after sequential extraction. The correlation coefficient (r) for (a) = -0.19 (b) = +0.26 (c) = -0.49 (d) = -0.13 (e) = -0.64 (f) = +0.21 and (g) = -0.51

The dissolution of the pectin without degradation can only happen at shorter exposure to the AAC; otherwise, prolonged exposure removes the PGA, hence lowering pectin molecular weight (Faravash and Ashtiani, 2007). Therefore, the increase in pectin yield with an increase in the AAC

for shorter extraction times of anthocyanins (Figure 15c) was facilitated by the intensification of the breaking of strongly bound pectin fractions, thereby promoting pectin solubilisation and recovery.

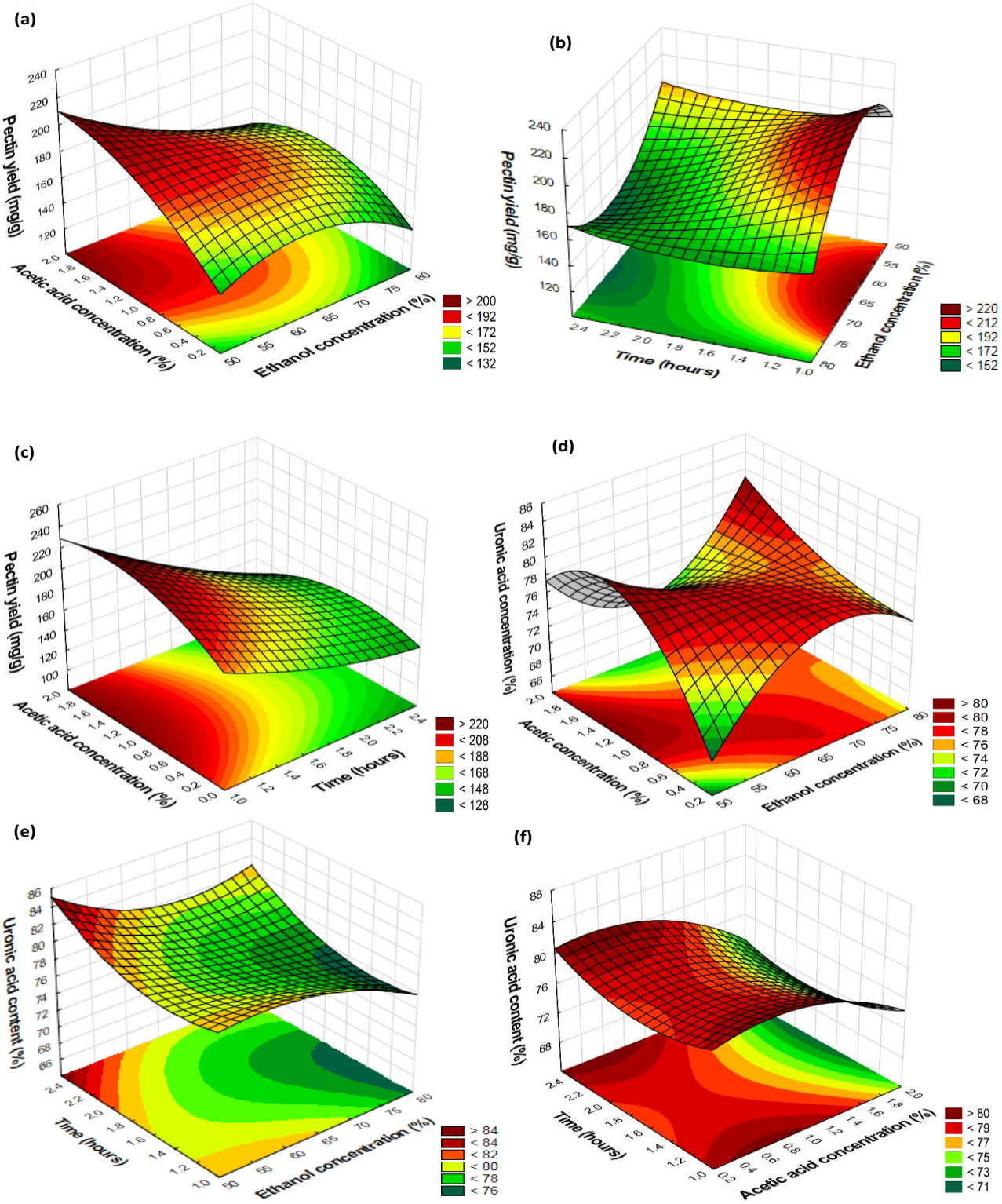

Figure 18: Response surface plots for the effects of anthocyanin extraction conditions on pectin properties

Conversely, the pectin UAC reduced with an increase in EC (Figure 18d and e), which is in agreement with Klinchongkon *et al*. (2016). Ethanol solubilises the water-insoluble part of the cell wall, thus, enabling the acidic extraction medium to penetrate and reach polysaccharide chains far within the cell walls (Galanakis *et al.*, 2010).

The quality of pectin source is based on the yield, UAC and DE (Raj *et al.*, 2012). The pectin recovered had a DE of about 70%, and none of the investigated anthocyanin extraction conditions had a significant effect on the DE ($p > 0.05$). Inherently, the high degree of esterification (DE > 50%) of native mango peel pectin inhibits the demethoxylation of the pectin by acid hydrolysis (Krall and McFeeters, 1998).

4.5.3. Optimisation of the sequential extraction of anthocyanins and pectin

Although pre-extraction of anthocyanins with ECs between 50–70% and AAC higher than 1.2% resulted in the highest anthocyanin (Figure 18a) and pectin yield and, the purity of the pectin and the antioxidant activity of the anthocyanins were reduced. Therefore, to obtain pectin and anthocyanins with the highest purity and antioxidant activity respectively an EC in the range of 65–80% and AAC higher than 0.4% should be used for the extraction of anthocyanins before pectin extraction.

Application of the Derringer's desired function (Boudaoud *et al.*, 2006) to determine the SEP optimum extraction conditions for maximising the yield of anthocyanin and pectin, polyphenol and UAC contents, and minimising the IC_{50} values, yielded five second-order polynomial models Equation 6 to 10.

$$AY\ (mg/100g) = -1.788X_1 + 0.025X_1^2 - 170.439X_2 - 15.344X_2^2 + 1.19X_3^2 + 5.729X_1X_2 + 0.304X_2^2X_1 - 0.049X_1^2X_2 + 4.16X_2X_3 \quad (6)$$

$$AA\ (\mu g\ GAE) = -5.8169 + 0.24X_1 - 0.0017X_1^2 - 11.29X_2 - 1.1X_2^2 + 0.13X_3^2 + 0.39X_1X_2 + 0.01X_1X_2^2 - 0.0032X_1^2X_2 - 0.072X_1X_3 + 0.0006X_1^2X_3 + 0.57X_2X_3 \quad (7)$$

$$PC\ (mg/g) = -2041.47 + 70.32X_1 - 0.55X_1^2 - 142.98X_2 + 11.42X_2^2 + 717.44X_3 + 18.68X_3^2 + 4.13X_1X_2 - 0.45X_1X_2^2 - 0.02X_1^2X_2 - 25.07X_1X_3 + 0.2X_1^2X_3 + 6X_2X_3 \quad (8)$$

$$PY\ (mg/g) = -1958.71 + 68.92X_1 - 0.53X_1^2 + 427.08X_2 + 923.4X_3 + 13.54X_3^2 - 10.53X_1X_2 + 0.08X_1^2X_2 - 31.06X_1X_3 + 0.24X_1^2X_3 - 17X_2X_3 \quad (9)$$

$$UAC\ (mg/g) = -1398.971 + 70.40X_1 - 0.51X_1^2 + 2140.94X_2 - 273.74X_2^2 + 340.68X_3 + 89.18X_3^2 - 56.23X_1X_2 + 4.04X_2^2X_1 + 0.36X_1^2X_2 - 19.97X_1X_3 + 0.15X_1^2X_3 - 10.69X_2X_3 \quad (10)$$

where:

AY = anthocyanin yield,

AA = antioxidant activity of the anthocyanin extract

PC = total polyphenol content of the anthocyanin extract

PY = pectin yield

UAC = uronic acid content

X_1, X_2 and X_3 = EC, AAC, and time, respectively.

The predicted optimum conditions were 67.45% w/w ethanol, 0.24% w/w AAC and 60 min extraction time. The conditions were validated experimentally, and the experimental and model prediction results are shown in Table 9. The experimental results were within the 95% response confidence level of the model predictions.

Table 9: Experimental results and model predictions of the pectin and anthocyanin properties at optimum conditions

	Pectin Properties			**Anthocyanins Properties**		
	Yield (AIR basis) (mg/g)	Uronic acid content (mg/g)	Antioxidant activity (%)	Anthocyanin yield (mg/100g)	Polyphenol content (mg/g GAE)	Antioxidant activity (µg GAE)
Experimental results	209.04	766	97.17	22.33	132.62	1.36
Model predictions	203.61	800	85.80	23.82	133.7	1.46

GAE; Gallic acid equivalent, AIR; Alcohol insoluble residue

4.5.4. Comparison of pectin properties at optimal sequential extraction to conventionally extracted pectin

The pectin yield at optimal conditions was 31% w/w higher (on a feedstock basis) (Table 9) and 23% w/w purer than pectin recovered from the CEP (Table 7). The DE, however, remained the same regardless of extraction conditions. Therefore, the results confirm that the pre-extracted residue can indeed be used as a raw material for extracting pectin of high purity and antioxidant activity, which would increase its economic value.

4.5.5. Structural properties of pectin extracted conventionally and sequentially at optimal conditions

The FTIR spectra for the CEP and SEP pectin were similar to that of the commercial pectin, confirming that the extracted biopolymer was pectin. The bands due to the stretching vibrations of the OH groups, C—H of the CH, CH_2 and CH_3 groups, C=O of carboxylic acid methyl ester, C=O of free carboxyl groups and the fingerprint region respectively (Pasandide *et al.*, 2017; Banerjee *et*

al., 2018) were visible (Figure 19). The differences in the content of C=O of carboxylic acid methyl ester and C=O of free carboxyl groups is quite clear between spectra (a) and (b) and between spectra (b) and (c) (Figure 19),which confirms the quality differences in terms of pectin UAC.

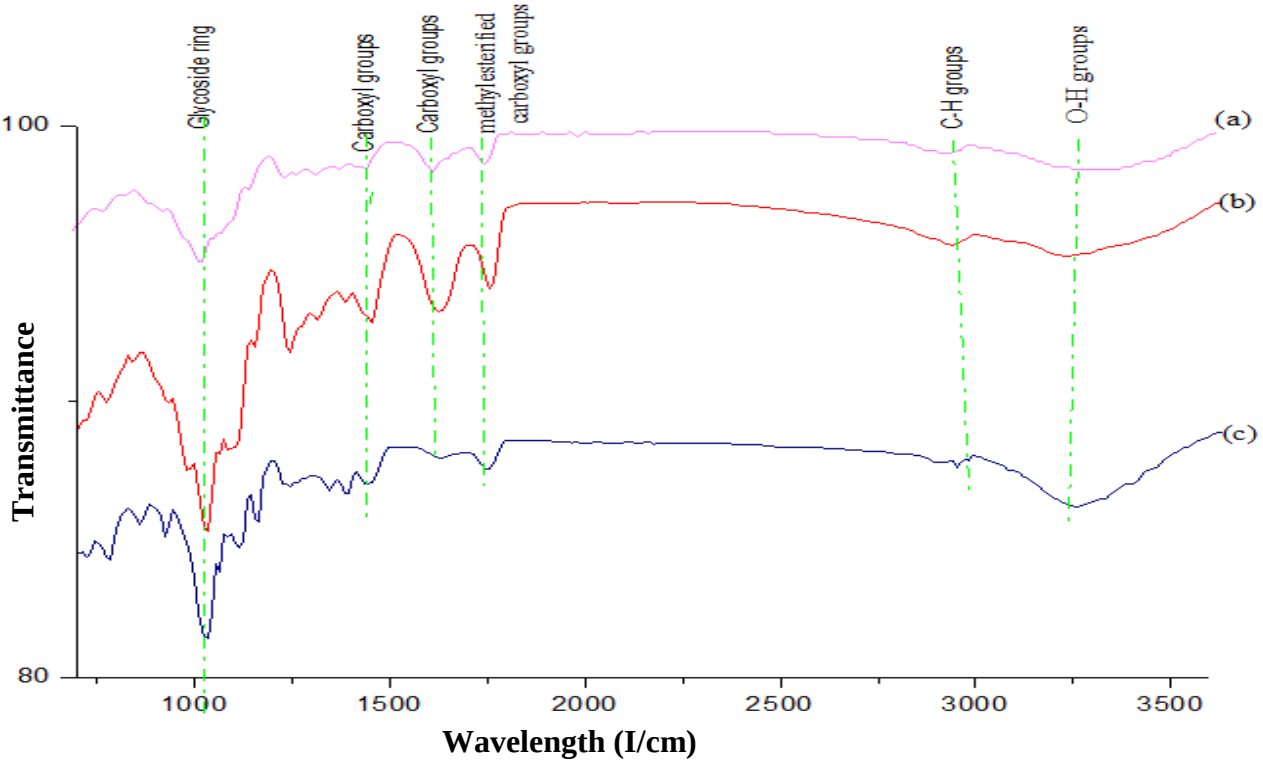

Figure 19. FTIR spectra of (a) pectin from the conventional extraction process, (b) pectin from the sequential extraction process and (c) commercial pectin

4.6. Conclusions

Despite differences in pectin and anthocyanins optimal extraction conditions, the proposed SEP was capable of recovering functional pectin and anthocyanins. The pectin was richer in PGA and had higher antioxidant activity than pectin from CEP. The increased purity and bioactivity of pectin are two important factors that would enhance its potential use in the pharmaceutical (as a drug carrier) and food packaging (food contact edible film) industries. At optimal conditions, the SEP yielded 209.04 mg/g pectin on an AIR basis, and the pectin had an antioxidant activity of 97.17% and a UAC of 766 mg/g. Thus, the integration of the SEP with already available anthocyanin or pectin extracting plants would enable the realisation of the full value potential of the mango peel. Similar approach will be used to add value to other fruit wastes rich in these components. Furthermore, the functionality of the pectin will be tested as a component in functional foods and in biocomposites materials for manufacturing active food packaging that exhibit antioxidant properties.

4.7. Paper Title: "Mango peels biorefinery: An investigation into sequential fractionation of mango peels for anthocyanins, polyphenols, and pectin".

Submitted to the journal of Biofuels, Bioproducts and Biorefining (impact factor 4.528)

4.8. Abstract

Multiple stages in biorefineries can negatively affect properties of co-products due to different optimal extraction conditions. The study investigated the effects of incorporating a polyphenol extraction stage in an anthocyanin-pectin sequential extraction process from mango (*Mangifera indica* L.) peels. Anthocyanins, the most labile, were extracted first with 80% ethanol (EC)/2% acetic acid at a temperature of 25 ^{o}C for 1 h, generating a residue from which polyphenols (second most labile) were extracted with EC ranging from 65–85% for a time range of 20–80 min at 70 ^{o}C, based on a full factorial statistical experiment. Pectin was subsequently extracted from the second stage residues with 0.25% ammonium oxalate/oxalic acid at 85 ^{o}C for 1 h. Anthocyanin extract had an anthocyanin content, polyphenol content and antioxidant activity of 38.87 mg/100 g, 113.2 mg/g and 0.62 µg GAE, respectively. The optimum polyphenol conditions (75% EC/60 min/70 ^{o}C) gave a polyphenol extract with a polyphenol content of 98.68 mg/g and antioxidant activity of 1.32 µg GAE. In addition, pectin extract (179 mg/g) with UAC of 855 mg/g and antioxidant activity of 84.69% was obtained from the residues. The polyphenols extracted at optimum conditions had a polyphenol content 28% lower and an antioxidant activity 65% higher than polyphenols extracted directly from the peels. Pectin recovered after the anthocyanin and polyphenols extraction had a 38% higher purity than the pectin from the SEP. Thus, extraction conditions can be manipulated to increase the number of functional high-value products in a mango peel-based biorefinery.

Keywords: Polyphenols ; Pectin; Anthocyanin; Mango peels; Sequential extraction; Full factorial design.

4.9. Introduction

Pectin, polyphenols and anthocyanins have recently gained attention as food ingredients and as additives to food packaging material for increased food shelf life (Adilah *et al.*, 2018). Pectin, a biopolymer consisting mainly of GA units has been utilised in developing films for food packaging because of its excellent barrier properties to lipids, moisture, and gases. On the other hand, due to the high antioxidant activity of polyphenols and anthocyanins, they are added to food and food packaging material to minimise the accumulation of agents responsible for food spoilage (free radicals and microorganisms), thereby increasing food shelf life (Espitia *et al.*, 2014; Adilah *et al.*, 2018).

The desired functional properties of anthocyanins, pectin, and polyphenols can be manipulated during their extraction (Pasandide et al. 2017; Elnour et al. 2018). Processes for the recovery of single products and multiple products have been developed and optimised with the aim of effectively extracting these products. However, multiple product extraction approaches such as co-extraction and sequential extraction have gained more popularity in the past decade (Rojas et al. 2015; de Souza et al. 2018). Co-extraction has been applied to recover polyphenols and pectin from mango peels. The process involved a single stage procedure consisting of a Amberlite XAD-16 resin which absorbed polyphenols either before or after precipitation of pectin (Berardini, Knödler, *et al.*, 2005; Rojas *et al.*, 2015; Banerjee *et al.*, 2018). The major drawback of this process was that it caused substantial degradation of polyphenols.

The co-extraction of anthocyanins, polyphenols, and pectin is limited by the differences in the optimum extraction temperatures required for the effective recovery of each of the products. Anthocyanins are generally extracted at temperatures below 50 ^{o}C to avoid degradation (Cacace and Mazza 2003; Hutabarat et al. 2019; Le et al. 2019). However, the optimum temperature range for anthocyanins is not sufficient for recovery of polyphenols and pectin. Polyphenols are extracted at temperatures > 70 ^{o}C and pectin at temperatures > 85 ^{o}C (Koubala et al. 2008; do Nascimento Oliveira et al. 2018). Furthermore, polyphenols were observed to degrade when co-extracted with pectin at 90 ^{o}C (Berardini et al. 2005). In addition, pectin yields are lower at temperatures < 80 ^{o}C (Mugwagwa and Chimphango, 2019). The differences in temperature requirements for the recovery of these products, could suggest that a multistage sequential extraction process will be effective in recovering the labile anthocyanins, polyphenols, and pectin (three-stage extraction) from mango peels. Multiple stage sequential extraction of phenolics and pectin has been extensively studied in other fruit waste such as apple pomace, orange peels and passion fruit rind (Schieber *et al.*, 2003; Boukroufa *et al.*, 2015; de Souza *et al.*, 2018). However, in most of these studies, the focus was on determining the yield and the properties of the recovered products without taking into consideration

the effect each extraction stage has on the properties of the products in the subsequent stages. Thus, the optimisation of the extraction conditions for the subsequent stages has not been done.

There has been a recent drive to sequentially extract anthocyanins and pectin from fruit waste (Garg *et al.*, 2019; Mugwagwa and Chimphango, 2019). The anthocyanin extraction in these processes recovered the loosely bound polyphenols in the mango peels because of the mild conditions (with temperatures as low as 25 ^{o}C) used (Irakli *et al.*, 2018; Mugwagwa and Chimphango, 2019). In the process, the tightly bound and thermally stable polyphenols were left exposed such that repeating the extraction of the polyphenols from the anthocyanin-free residue at elevated temperatures, is likely to facilitate the recovery of the tightly bound polyphenols as an additional product stream. Furthermore, the conditions applied in the polyphenol extraction step (stage 2) can remove neutral sugar side branches in pectin, thereby increasing the purity of the pectin recovered in the subsequent process (Pasandide *et al.*, 2017), which can favour the application of pectin in the food industry. Therefore, the incorporation of a polyphenol extraction stage after anthocyanin extraction can increase the number and functionality of products that can be recovered from mango peels.

In this study, a three-stage extraction process involving a two-step ethanol-based extraction of phenolics (anthocyanins and polyphenols) prior to pectin extraction was developed. Polyphenol extraction from the anthocyanin free residue was optimised taking into consideration the properties of pectin (yield, UAC, polyphenol content, neutral sugars content, antioxidant activity) remaining in the residue. The aim was to increase the number of co-products obtained from the mango peels that can be used as additives to food and food packaging material, from two (anthocyanins, and pectin) to three products (adding polyphenols) with improved functional properties, in particular, improved purity for the pectin and increased antioxidant activity for the two phenolic fractions despite having different optimal extraction conditions. Pectin with higher purity is less branched, thus, more hydrophobic and has closely packed particles, hence, can be used in developing food packaging films with improved barrier properties (Yapo *et al.*, 2007). Polyphenols with a high antioxidant activity have the ability to scavenge free radicals and food spoilage microorganisms, thereby increasing the shelf life of food (Adilah *et al.*, 2018). The novelty of this work lies in sequentially extracting three products from mango peels although they have different optimum extraction conditions.

4.10. Materials and methods

The chemicals used for the experiments and their sources are listed in section 4.4.1. Mango peels were prepared prior to experiments as highlighted in section 4.4.1

4.10.1. Three-stage sequential extraction procedure

The sequential extraction stages are highlighted in Figure 20. Detailed descriptions of each stage are in the sections below.

4.10.1.1. First extraction stage

The anthocyanin extraction conditions were arrived at by optimising the anthocyanin extraction conditions highlighted in Mugwagwa & Chimphango, (2019) taking into consideration anthocyanin properties (yield and antioxidant activity), and the properties of polyphenols (yield and antioxidant activity) and pectin (yield, antioxidant activity and UAC) in the residue. The optimum anthocyanin conditions were 80% ethanol/2% acetic acid, reaction time of 1 h at 25 ^{o}C. Anthocyanins were extracted based on the method in Mugwagwa & Chimphango, (2019). Mango peels (200 g) were homogenised with a 4 L solution of 80% ethanol/2% acetic acid in Schott bottles for 1 min using a high speed blender (220–240 V, 50 Hz, 250 W). Thereafter the mixture was stirred at 800 rpm using a magnetic stirrer for 1 h at 25^{o}C to extract the anthocyanins. An anthocyanin rich liquid was obtained by centrifuging the mixture at 8000 rpm for 20 min. The anthocyanin extract was stored at -4 ^{o}C in plastic tubes wrapped with aluminium foil. The remaining solid residue (75.6 g) was washed with water to remove any traces of anthocyanins and dried for 3 days at 40 ^{o}C in an oven.

4.10.1.2. Second extraction stage

The anthocyanin-free residue from stage 1 was the feedstock for extraction of polyphenols. A full factorial design with EC (65–85%), time (20–80 min) and a temperature of 70 ^{o}C was adapted for extraction of polyphenols. Approximately 6 g of the mango residue was added to 50 mL of ethanol with concentrations highlighted in Table 10. The polyphenol extraction was carried out in Schott bottles in a shaking water bath based on the experimental conditions in Table 10. Thereafter the residue (AIR) was thoroughly washed to remove any traces of polyphenols and oven-dried at 40 ^{0}C for 2 days to a moisture content < 10%.

4.10.1.3. Third extraction stage

The residue from the second stage was subjected to ammonium oxalate/oxalic acid-based pectin extraction as previously described by Koubala *et al*., (2008). Approximately 4 g of the residue was reacted with 160 mL of 0.25% ammonium oxalate/oxalic solution at a pH of 4.6. The reaction was carried out in Schott bottles incubated in a shaking water bath set at 85 ^{o}C for 1 h. A pectin rich supernatant (approximately 140 mL) was recovered after vacuum filtration (45 µm filter paper) to remove the solid material. The pectin was then precipitated with 95% ethanol and allowed to settle for 3 h before being skimmed out. The precipitated pectin was thoroughly washed with 70% ethanol and then finally with 90% ethanol prior to oven drying at 40^{o}C for 3 days.

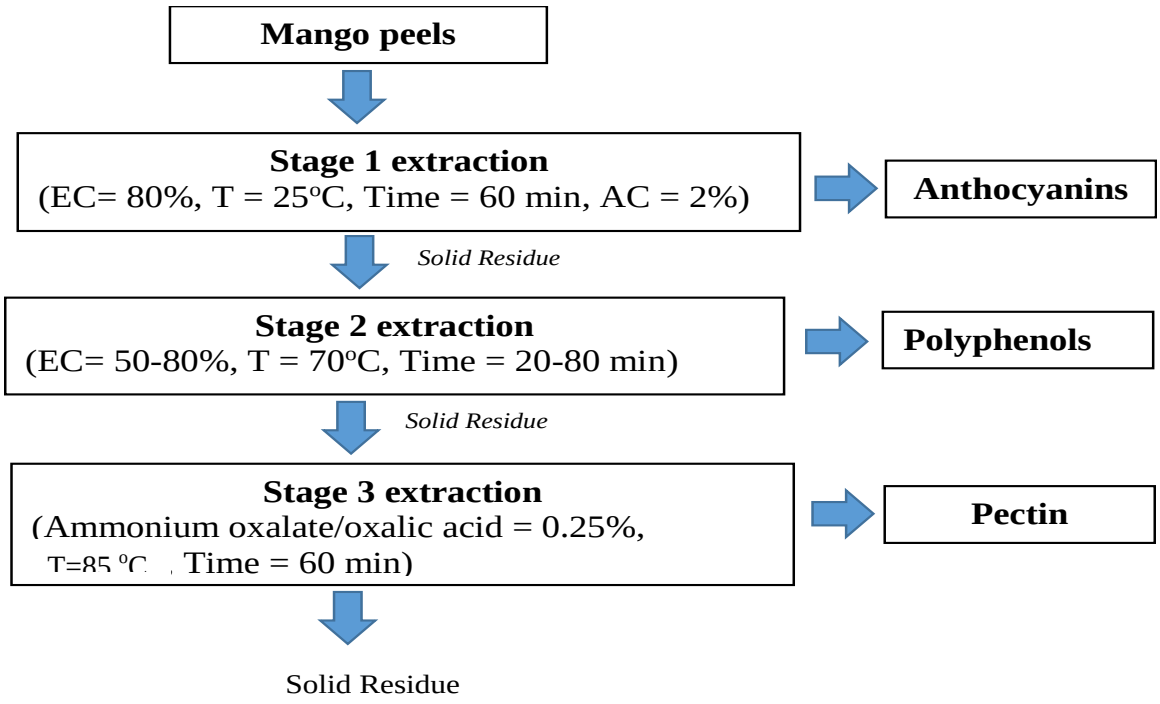

Figure 20: Process flowsheet for the multistage fractionation of mango peels. T: Temperature, EC: Ethanol concentration, AC: Acetic acid concentration

4.10.2. Two-step extraction of polyphenols and pectin from mango peels

Polyphenols were extracted directly from the mango peels without removal of anthocyanins with 75% ethanol for 60 min (in three 20 min cycles) in a shaking water bath maintained at a temperature of 70 °C (Figure 21). Pectin was extracted then extracted from the residues remaining after polyphenol extraction with 0.25% ammonium oxalate/oxalic acid solution at 85 °C for 60 min (Figure 21).

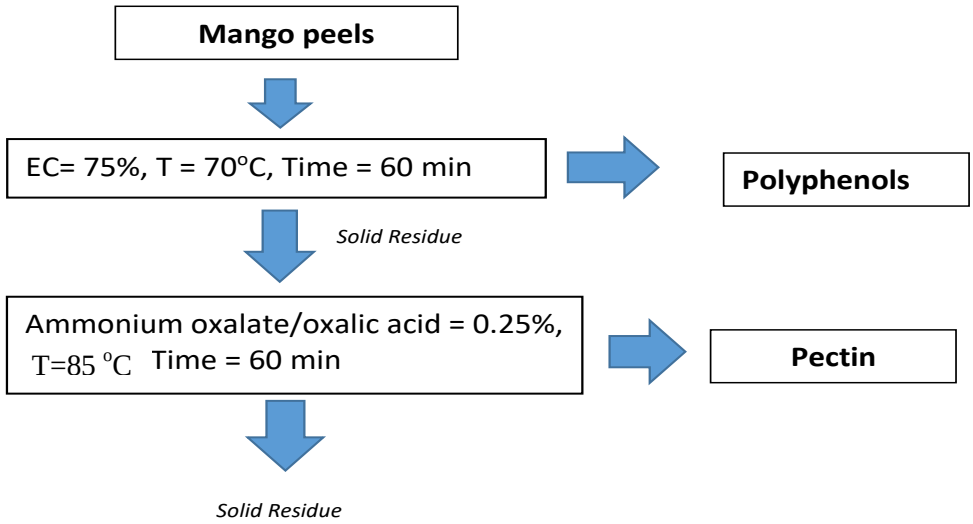

Figure 21: Two-step extraction of polyphenols and pectin from mango peels. T: Temperature, EC: Ethanol concentration

4.10.3. Characterisation of anthocyanins, polyphenols, and pectin.

The anthocyanins and polyphenols yield and antioxidant activity was determined based on the methods described in section 4.4.3. Pectin yield, neutral sugars content, UAC polyphenol content and antioxidant activity was determined according to the methods in sections 4.4.6 to section 4.4.8. The chemical bonding in pectin was analysed by the Fourier transform infrared spectroscopy as explained in section 4.4.9.

4.10.4. Experimental design

A full factorial experimental design (3^2) with the extraction conditions in Table 10 was applied to determine the effects of the polyphenol extraction conditions on the polyphenol and pectin properties. Optimisation of the polyphenol extraction conditions was performed using the Derringer's desired function methodology. All analyses were done in triplicate. The experimental design and analysis of results was performed using the software Statistica 7 version 13.2.

Table 10: Polyphenol extraction conditions

	Process conditions	
Run	Ethanol concentration (%)	Time (min)
1	65	20
2	65	60
3	65	80
4	75	20
5	75	60
6	75	80
7	85	20
8	85	60
9	85	80

4.11. Results and discussions

4.11.1. Effect of pre-extraction of anthocyanins (stage 1) from mango peels on the functional properties of polyphenols recovered in stage 2

An anthocyanin extract with anthocyanin content of 38.87 mg/100g and a polyphenol content of 113.20 mg/g was recovered from the mango peels in stage 1 of the three-stage sequential extraction process. Residues remaining after stage 1 were approximately 37.8% of the initial mango peels and contained up to 122.8 mg/g polyphenols (Table 11). Fractionation of stage 1 residues resulted in the recovery of polyphenol extracts (stage 2) with higher polyphenol content than the anthocyanin extract (Table 11). Similar results were reported by Garcia-mendoza *et al*., (2015) when they recorded higher polyphenol yields in the second polyphenol extraction stage than the first stage in a sequential process (Garcia-mendoza et al., 2015).

The differences in polyphenol content in the two extracts can be attributed to the differences in temperatures applied in the two extraction stages (Figure 20). A direct correlation between polyphenol solubilisation in ethanol and temperature has been reported in previous research. For example, the findings of Xu *et al*., (2014) showed that increasing temperature from 30 ^{o}C to 80 ^{o}C during thermal treatment of citrus peels, boosted the recovery of phenolic acids (Xu et al., 2014). The low temperature (25 ^{o}C) applied in stage 1 may have facilitated the recovery of the loosely bound polyphenols, leaving the tightly bound polyphenols in the residues. The use of a higher temperature (70 ^{o}C) in stage 2 unlocked the tightly bound polyphenols promoting their recovery (Su *et al.*, 2014). Furthermore, the increased yield can also be attributed to the interaction between the high temperature (70 ^{o}C) and the high EC (75%) broke the phenolics-polysaccharides bonds resulting in the release of polyphenols from mango peels (Shi *et al.*, 2003).

Interestingly, the combined total quantities of polyphenols recovered in the three-stage sequential process (stage 1 and stage 2) exceeded (>163 mg/g) that of a polyphenol extract recovered directly from the mango peels (136.23 mg/g) (Table 12). The single-step recovery of polyphenols may have resulted in the degradation of polyphenols, particularly, the anthocyanins due to the high extraction temperature (70 ^{o}C). Therefore, the two-stage polyphenol extraction process not only increase the number of products that can be recovered from mango peels but reduces their degradation.

More importantly, the polyphenol extracts (stage 1 and stage 2 extracts) from the multistage extraction process had higher antioxidant activity (stage 1 was 0.62 µg GAE and for stage 2 ranged from 0.56 µg to 2.23 µg GAE) than the polyphenol extract (3.75 µg GAE) from the single-step extraction process (Table 11 and 12). Therefore, the pre-extraction of anthocyanins before extracting polyphenols extraction is an effective method of obtaining polyphenol extracts with higher antioxidant activity than the single-stage extraction process with potential application as food colourants and antioxidants. This implies that lower dosages of polyphenols from the three-stage can be incorporated into food and have better scavenging capabilities for food spoilage agents than polyphenols from a single-stage extraction process. This is an important property for food additives as there are regulations on concentrations of additives that can be added to food (EU, 2011).

Table 11: Yield and composition of polyphenol and pectin extracted at different extraction conditions post anthocyanins extraction

Anthocyanins				Polyphenol extract		AIR	Pectin				
Anthocyanin yield (mg/100g)	Polyphenol content (mg/g GAE)	Antioxidant activity (µg GAE)	Anthocyanin-free residue remaining (%)	Polyphenol yield (mg/g)	Antioxidant activity (µg GAE)	AIR (%)	Yield (AIR basis) (mg/g)	Uronic acid content (mg/g)	Neutral sugars content (mg/g)	Polyphenol content (mg/g)	Antioxidant activity (%)
38.87	113.20	0.62	37.8								
				122.80	0.56	81.49	166.28	829.63	13.90	20.92	86.29
				101.39	1.22	81.30	167.50	858.59	15.18	22.27	79.49
				50.65	1.99	80.09	167.43	818.65	123.84	24.21	78.49
				113.70	1.06	81.03	161.58	868.98	43.91	24.11	81.64
				94.22	1.55	85.60	162.40	882.36	45.50	24.47	82.64
				59.41	1.93	81.82	159.50	831.03	45.81	25.60	88.03
				75.84	1.92	85.57	160.25	790.29	45.45	24.63	94.71
				65.05	1.98	79.99	164.35	807.86	107.12	23.81	81.94
				51.78	2.23	79.18	158.58	783.90	115.65	25.68	81.60
P-Values (3 decimal places)											
(1) EC				0.01	0.00		0.03	0.00	0.44	0.01	0.66
(2) T				0.00	0.00		0.22	0.02	0.31	0.02	0.48
1*2				0.02	0.00		0.44	0.08	0.65	0.09	0.68

AIR: Alcohol insoluble residue; GAE: Gallic acid equivalent; EC: Ethanol concentration; T: Time

4.11.2. Effect of polyphenol extraction conditions (stage 2) on the yield and functional properties of polyphenols (stage 2) and pectin (stage 3)

The properties of the polyphenol extract recovered in stage 2, namely, polyphenol content and antioxidant activity were significantly ($p < 0.05$) affected by EC and extraction time (Table 11). Thus, it is important to control these extraction conditions and their interactions to obtain functional polyphenols. Fractionation of the anthocyanin-free residue with lower EC, that is decreasing EC from 85% to 65%, for shorter periods (reducing extraction time from 80 min to 20 min), facilitated extraction of polyphenol extracts with higher polyphenol content (Figure 22a) and antioxidant activity (Figure 22b) than extended exposure to higher EC. Therefore, the residue after anthocyanin extraction presents an economic raw material for extracting polyphenols as it requires lower EC (65% vs 75%) and shorter extraction time (20 min vs 60 min) (Figure 22) than extracting polyphenols directly from the mango peels (Table 12).

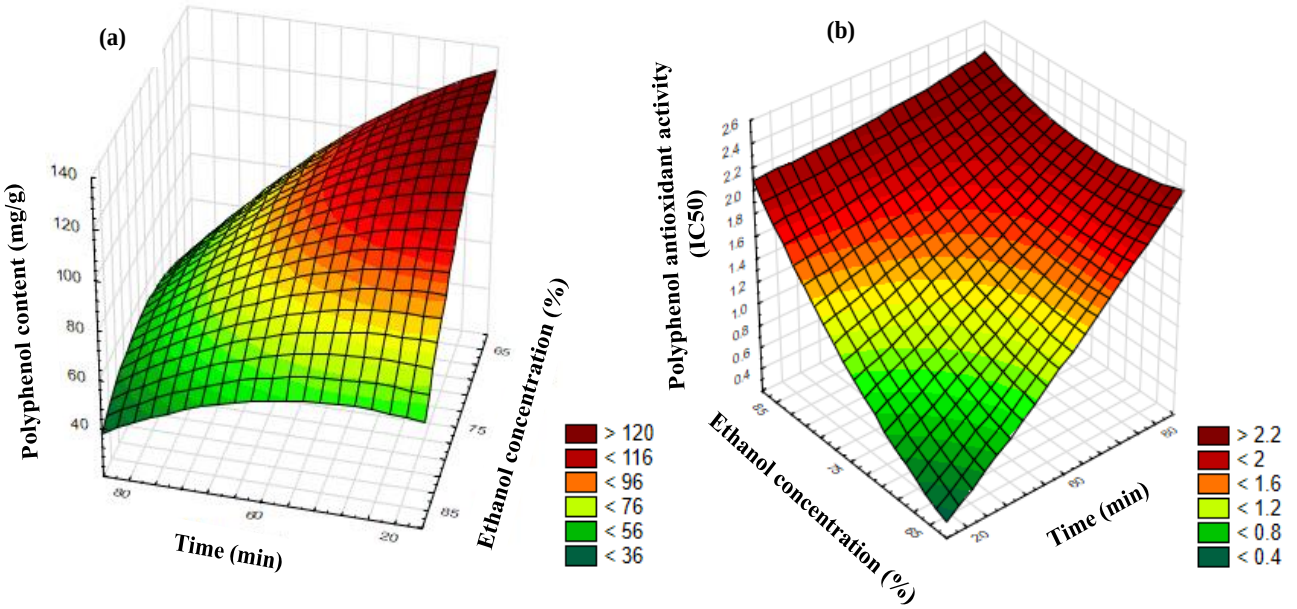

Figure 22: Response surface plots for the effects of polyphenol extraction conditions on polyphenol properties

The polyphenol extraction stage, stage 2, left a residue (AIR) of approximately 79.18 - 85.60% of the anthocyanin-free residue (Table 11) depending on the polyphenol extraction conditions used. Pectin recovered from the AIR ranged from 167.5 - 158.58 mg/g, and had a UAC ranging from 783.90 - 882.36 mg/g. The pectin was of higher purity and quality (GA content > 650 mg/g) (Table 11) when compared to pectin from the two-step polyphenol-pectin extraction process (Table 12). In addition, the three-stage extraction process enhanced the pectin purity beyond the purity recorded for pectin recovered from the two-stage sequential extraction of anthocyanins and pectin (Mugwagwa and Chimphango, 2019). Therefore, the three-stage extraction process effectively increased mango peels utilisation and in the process enhanced the properties of the extracted products. More importantly, the process can be used to recover pectin and polyphenols, from the

same feedstock, that can be integrated to develop food packaging material with excellent barrier properties (due to high purity of pectin) and active properties (high antioxidant activity of polyphenols).

Pectin yield was significantly affected by only EC ($p < 0.05$) used in stage 2. Higher yields of pectin were recovered as EC decreased from 85% to 65% (Figure 23a). Maximum yields were obtained at an EC of 65% and a time of 60 min. The same solvent concentration yielded polyphenols with the highest polyphenol content and antioxidant activity. Therefore, maintaining the EC at 65% is conducive for extracting high yields of pectin and polyphenols. However, at this EC the purity of pectin, measured as UAC, was at its lowest, (Figure 23b) even though the pectin polyphenol (Figure 23c) and neutral sugars content (Figure 23d) were at their lowest values. This might be because the higher water content in the polyphenol extraction medium at lower EC promoted solubilisation of the PGA chain of the pectin resulting in the loss of pectin (Raj *et al.*, 2012). Therefore, low EC limits the quantity of pectin that can be recovered, thereby affecting the application of pectin as a food additive and/ or its use in the formulation of packaging films.

Uronic acid and polyphenol contents of the pectin were significantly affected ($p < 0.05$) by EC and extraction time in stage 2 (Table 11). The polyphenol extraction conditions that preserved uronic acids (increase in EC from 65% to 75%) (Figure 22b) and polyphenols in the pectin (Figure 22c) reduced the neutral sugars content (Figure 23d) and antioxidant activity (Figure 23e) of the pectin. This implies that it is possible to obtain pectin with higher purity but it will be at the expense of the pectin's antioxidant activity. Therefore, it is possible to manipulate the two-stage polyphenol extraction process in order to customize pectin properties, with respect to purity and antioxidant activity, depending on the end application. A two-stage ethanol-based phenolics extraction process prior to pectin extraction facilitates the extraction of polyphenols and anthocyanins with high antioxidant activities, at the same time the conditions can be used to increase pectin purity. The three-stage extraction process can be used in recovering pectin and bioactive compounds suitable for developing active packaging material.

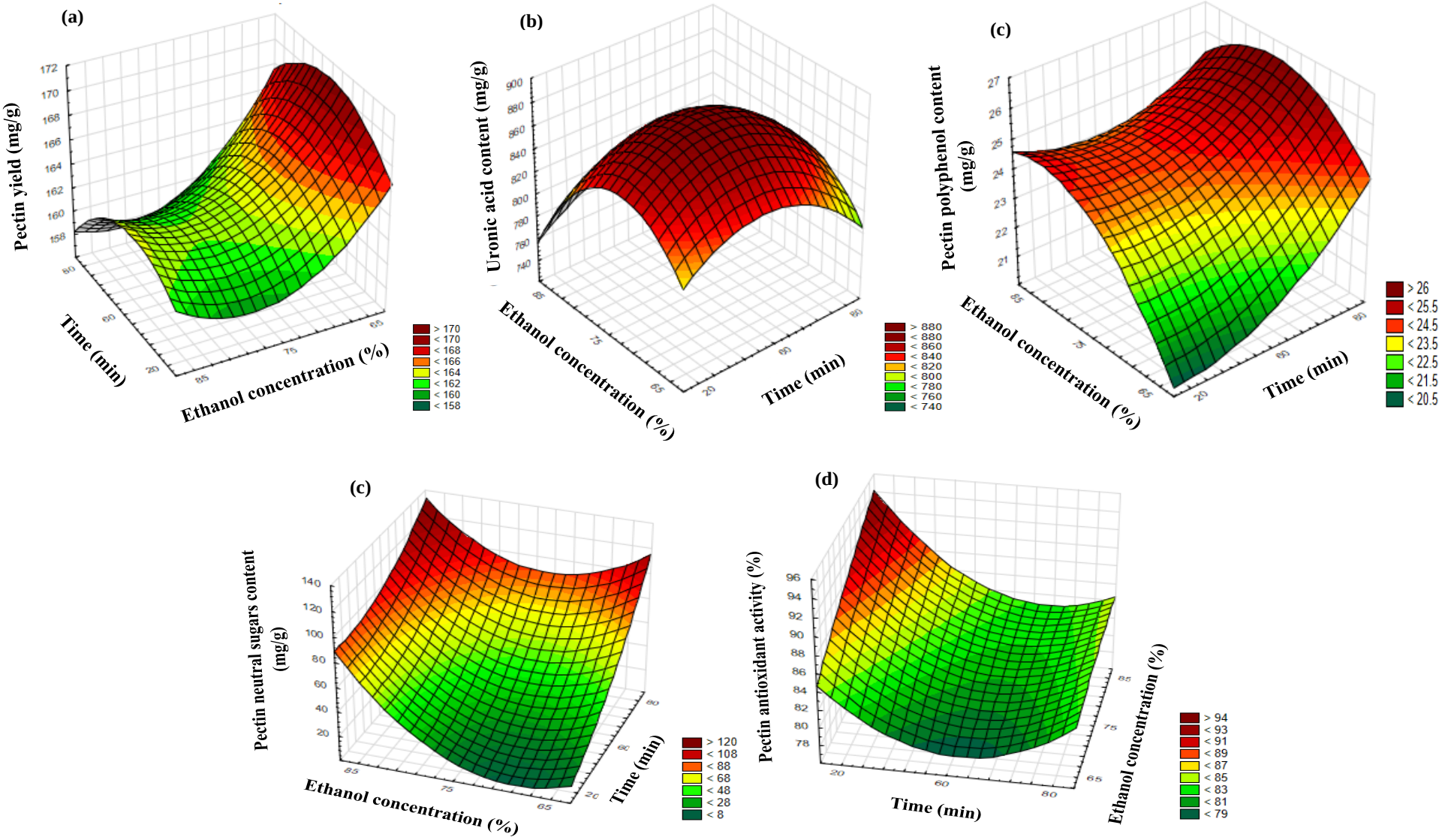

Figure 23: Response surface plots showing the effects of the polyphenol extraction conditions on pectin properties

4.11.3. Correlating the properties of polyphenols from stage 2 and pectin from stage 3

In a biorefinery set up, information on the effects of extraction stages on the products recovered in a multiple stage extraction process is important to determine the feasibility and viability of a multi-product process. The polyphenol extraction conditions (stage 2) that resulted in a polyphenol extract with a high polyphenol content promoted high pectin yields in stage 3, as depicted by the positive correlation in Figure 24a. A negative correlation was observed in the polyphenol content of pectin (stage 3) and the polyphenol extract (stage 2) (Figure 24b), confirming that the conditions in stage 2 were well suited for the removal of the polyphenols that were bound to the pectin. As more polyphenols were extracted, the UAC of the pectin increased, which is an indication of increased purity (Figure 24c). However, the removal of the polyphenols from pectin, reduced the pectin antioxidant activity as highlighted by the negative correlation in Figure 24d. Therefore, in order to improve the purity of the pectin, polyphenols have to be recovered from mango peels in higher quantities prior to pectin extraction, thus increasing the number of high value products that can be recovered from the mango peels.

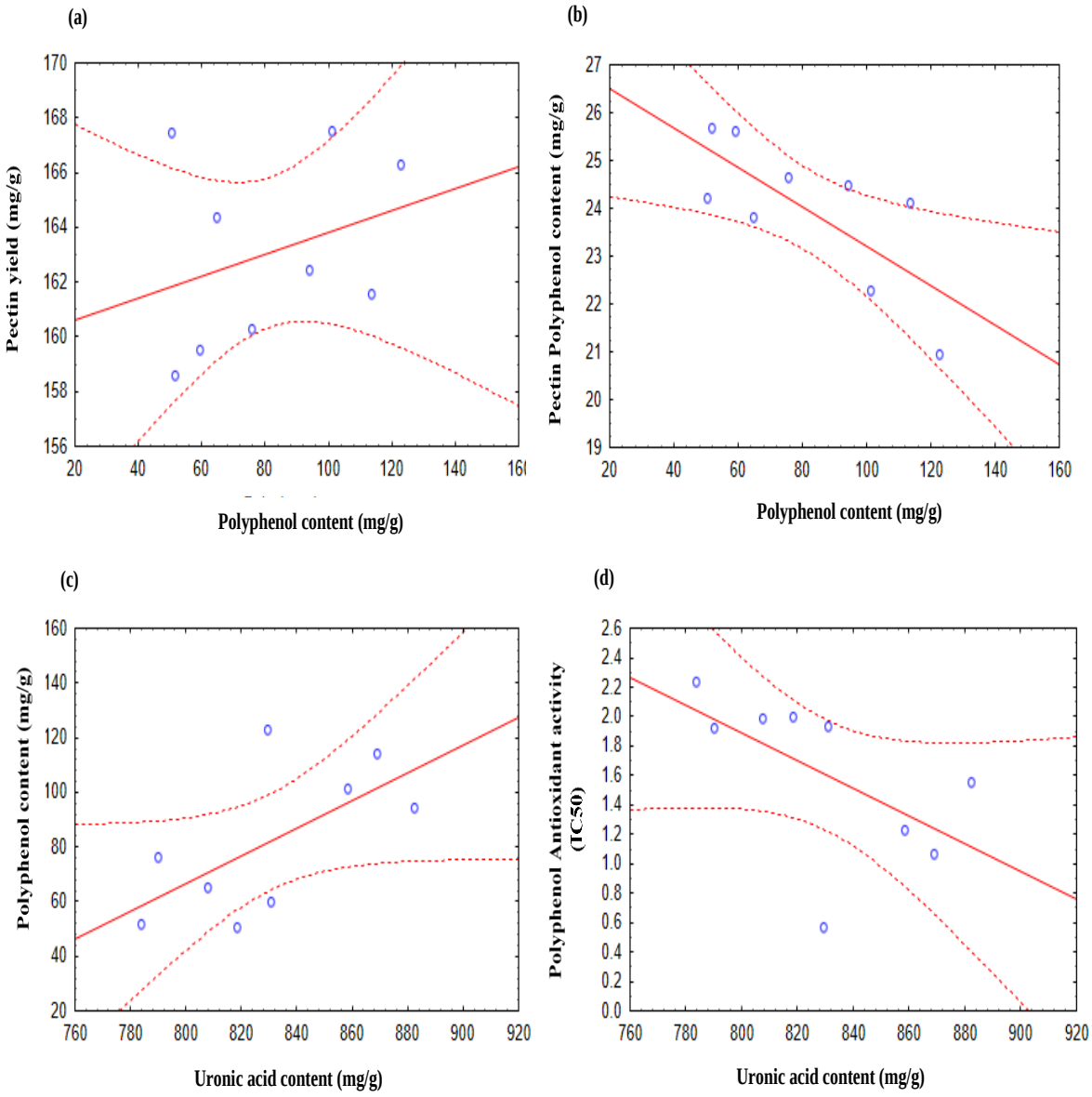

Figure 24: Correlations of polyphenol and pectin properties. The correlation coefficient (r) for (a) = +0.32 (b) = -0.74 (c) = +0.64 and (d) = -0.59

There was a positive correlation between the polyphenol content of the pectin and the antioxidant activity of the pectin (Figure 25a) suggesting that there is need to preserve the polyphenols in the pectin extract, thereby if the pectin is to be used as a bioactive polymer (Mugwagwa and Chimphango, 2019). Similarly, the antioxidant activity of the polyphenol extract, recovered from stage 2, increased with an increase in the polyphenol content of the polyphenol extract (Figure 25b). However, this contradicts the work by Berardini *et al*., (2005), who found no correlation between the polyphenol content and antioxidant of mango peel polyphenol extracts.

The presence of neutral sugars in the pectin had an inhibiting effect on the antioxidant activity of the pectin (Figure 25c), therefore, it is vital to reduce the neutral sugars content of the pectin to ensure high antioxidant activity. There was no correlation between polyphenol antioxidant activity and the antioxidant activity of the pectin (Figure 25d). Thus, the extraction of polyphenols with a

high antioxidant activity in stage 2 (Figure 25d) does not necessarily mean that the pectin extracted from the remaining residue will have reduced antioxidant activity (Figure 25a).

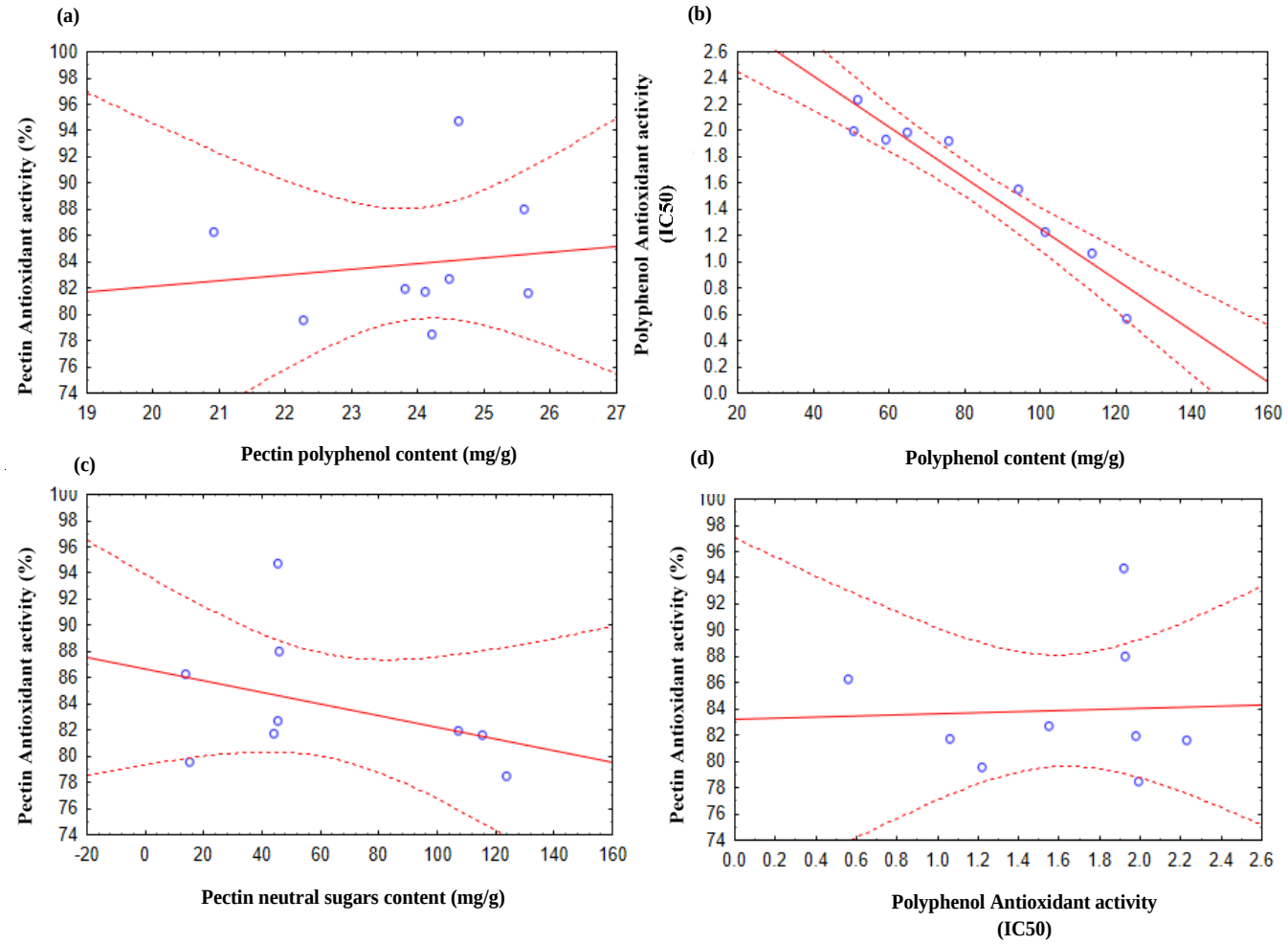

Figure 25: Correlation plots for the properties of the polyphenol extract (polyphenol content and antioxidant activity) and pectin (antioxidant activity, neutral sugar and polyphenol content). The correlation coefficient (r) for (a) = +0.13 (b) = -0.96 (c) = -0.37 and (d) = +0.04

The polyphenol extraction conditions were optimised, based on the model equations 11-15, by applying the Derringer's desired function methodology. The optimum conditions for polyphenol extraction (stage 2) were obtained by maximising the yield, UAC and antioxidant activity of pectin and the polyphenol content and antioxidant activity of the polyphenol extract (stage 2). In addition, the polyphenol and neutral sugars content of pectin were minimised. The relationships between the response variables and independent variables, Equations 11-15, were formulated taking into consideration only the significant factors.

$$Pectin\ yield\ (mg/g) = 17.13722 - 1.46208X_1 \quad (11)$$

$$Uronic\ acid\ content\ (\%) = 63.97 + 16.31X_1 - 4.60X_1^2 + 10.75X_2 - 2.91X_2^2 \quad (12)$$

$$Pectin\ polyphenol\ content\ (\%) = 15.98 + 6.81X_1 - 1.14X_1^2 \quad (13)$$

$$Polyphenol\ content(mg/g) = 134.75 - 10.49X_1^2 + 11.26X_1X_2 \quad (14)$$

$$Polyphenol\ antioxidant\ activity = 0.14X_1^2 + 0.87X_2 - 0.28X_1X_2 \quad (15)$$

Where X_1 is EC and X_2 is time

The optimum conditions for polyphenol extraction in stage 2 were EC of 75% and a reaction time of 60 min. The properties of the polyphenol extract and pectin were within the 95% confidence interval of the model predictions. The model results and the experimental results are given in Table 12.

Table 12:Experimental results and model predictions of the properties of polyphenols and pectin

	Polyphenol extract (Stage 2)		**Pectin Properties (Stage 3)**		
	Polyphenol content (mg/g GAE)	Antioxidant activity (µg GAE)	Yield (AIR basis) (mg/g)	Uronic acid content (mg/g)	Antioxidant activity (%)
Two-step	136.23 ± 5.5	3.75 ± 0.16	189.7 ±0.43	800.19± 6.46	92.48 ± 0.77
3-stage	98.68 ± 3.74	1.32 ± 0.04	179 ± 0.43	855 ± 0.32	84.69 ± 0.94
Model prediction	94.35	1.49	162.80	880.25	85.80

AIR: Alcohol insoluble residue, GAE: Gallic acid equivalent

The antioxidant activity of the anthocyanin extract (Table 11) was higher than that of the polyphenol extract obtained from the three-stage sequential extraction process at optimum conditions (Table 12). This might be due to the extraction of lipophilic polyphenol during the anthocyanin extraction stage leaving most of the hydrophilic polyphenols in the residue. The high EC (80%) and acid concentration (2%) used for anthocyanin extraction favours the solubilisation of most of the lipophilic polyphenols leaving most of the hydrophilic polyphenols in the residues. The

subsequent extraction of the polyphenols from the remaining residues at the reduced EC and, increased pH could have contributed to a polyphenol extract with lower lipophilic polyphenols but richer in hydrophilic polyphenols in stage 2. Lipophilic polyphenols have been reported to have higher antioxidant activity when compared to hydrophilic ones (Lizárraga-Velázquez *et al.*, 2018). Therefore, extraction conditions can be manipulated to obtain two polyphenol extracts with different properties.

4.11.4. Pectin structural and functional groups analysis

The FTIR spectra of the pectin extracted by the 3-stage extraction process at optimum conditions, using the two-step method and that of commercial pectin (CP) are compared in Figure 26 and Table 13. In addition, the assigned groups are compared in Table 13. Evidently, the spectra of the pectin extracted from the 3-stage extraction process and two–step method have a profile similar to that of the commercial pectin (Figure 26).

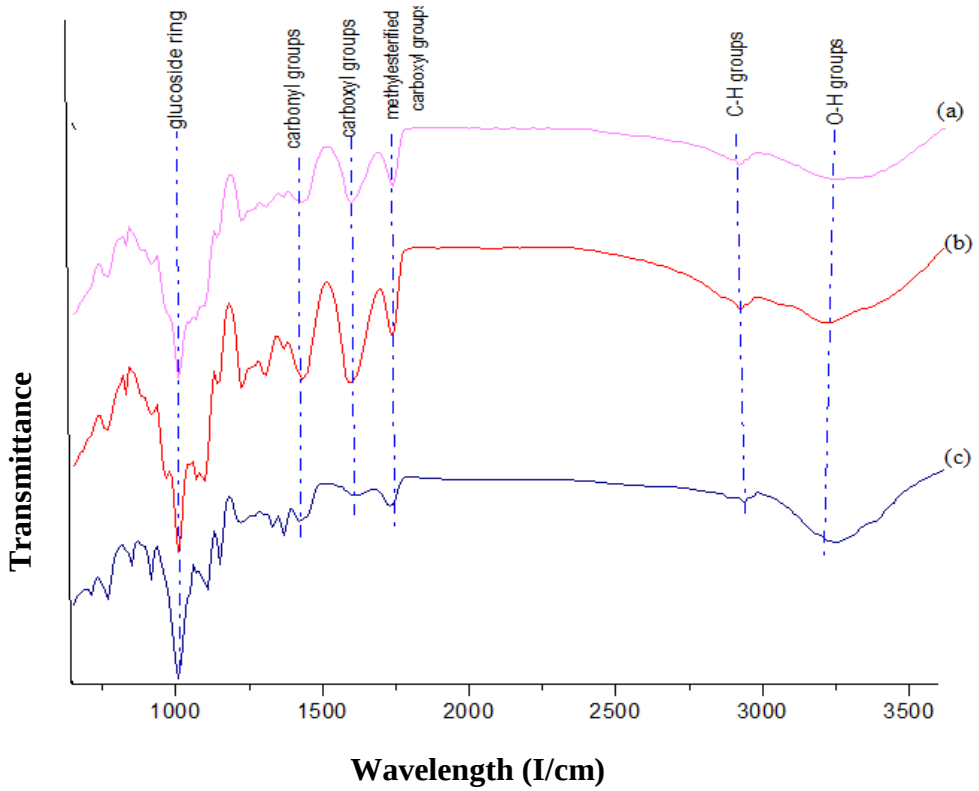

Figure 26: FTIR of the (a) pectin from the two-step extraction process, (b) pectin from the 3-stage extraction process and (c) commercial pectin

The peaks for the carboxyl groups in the spectra (Figure 26) for 3-stage extraction process were more pronounced than those for pectin from the two-step method and the commercial pectin, which is a reflection of a higher purity in the pectin extracted using the 3-stage extraction process than the pectin from the two-step method and the commercial pectin. The FTIR peaks of SEP at 3213.78cm^{-1}, 2925cm^{-1}, 1742.89 cm^{-1}, 1610.20 cm^{-1} and 800–1200 cm^{-1}, are due to the stretching vibrations of

the OH groups, C—H of the CH, CH_2 and CH_3 groups, C=O of carboxylic acid methyl ester, C=O of free carboxyl groups and the finger-print region respectively (Banerjee *et al.*, 2016; Pasandide *et al.*, 2017). The results confirm that the biopolymers extracted by both the 3-stage extraction process and two-step process are pectin as evidenced by their spectra peaks being similar to commercial pectin.

Table 13: Vibrational peaks of bonds in pectin

Two-step process	Three-stage process	CP	Vibrational Peaks
3258.39	3213.78	3241.45	O—H
2930.89	2925.81	2941.06	C—H
1740.07	1742.89	1734.98	C=O of methyl esterified carboxyl
1612.46	1610.20	1615.28	C=O of free carboxyl groups
800-1200	800-1200	800-1200	Fingerprint region

CP: Commercial pectin

4.12. Conclusions

This study has demonstrated that functional anthocyanins, polyphenols, and pectin with improved properties can be sequentially extracted from mango peels. An anthocyanin extract with anthocyanin content, polyphenol content and antioxidant activity of 38.87 mg/100g, 113.20 mg/g and 0.62 µg GAE, respectively, was recovered first. Optimisation of polyphenol extraction from the anthocyanin-free residue resulted in the optimum conditions of 75% EC and a time of 60 min. At these optimum conditions, a polyphenol extract with a polyphenol content of 98.68 mg/g and antioxidant activity of 1.32 µg GAE was recovered. Pectin with the following properties; pectin yield, 179 mg/g, UAC, 855 mg/g and antioxidant activity of 84.69% was recovered from the residue. In comparison to the two-step extraction process, the three-stage extraction process recovers a polyphenol extract and pectin with better functional properties. Hence, the three-stage extraction process does not only increase the number of products that can be recovered from mango peels but also improves their functional properties. The extraction of polyphenols with high polyphenol content and antioxidant activity improves the purity of pectin recovered from the residue, however, negatively affecting the pectin's antioxidant activity. Therefore, the three stage extraction process is a step towards full valorisation of mango peels, a fruit waste, for recovering value-added products that can be used as additives to food and food packaging material to increase food shelf life.

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5. SELECTIVE ORGANOSOLV PRE-TREATMENT OF WHEAT STRAW FOR ENHANCED HEMICELLULOSE HYDROPHOBICITY AND HEMICELLULOSE-FILLER INTERACTION

Titles of papers contributing to the chapter

1. Optimising alkali-based organosolv pre-treatment of wheat straw to improve hydrophobicity and filler interaction during acetylation of hemicellulose.

Dissertation objectives fulfilled in this chapter

This chapter seeks to fulfill objective 2 of the study. Hemicellulose-based films' mechanical properties, hydrophobicity and compatibility with fillers can be tailor-made by manipulating the biomass pre-treatment processes prior to hemicellulose extraction. The effects of pre-treating wheat straw before hemicellulose extraction on hemicellulose-based films acetylation, film hydrophobicity and mechanical properties, were investigated. Two optimum pre-treatment conditions were obtained, which were, one for enhancing hydrophobicity and the other which favoured hemicellulose acetylation and mechanical properties of the AH films.

Summary of findings

Pre-treatment significantly affected hemicellulose modification by acetylation and hemicellulose-based films' hydrophobicity and mechanical properties. Severe pre-treatment conditions promoted hydrophobicity of films, and reduced the acetylation DS required to achieve water resistivity in films, however, the film's mechanical properties were negatively affected. Milder pre-treatment processes enhanced the acetylation of hemicellulose and mechanical properties of the films but negatively affected film hydrophobicity. Alkali-based organosolv pre-treatment of biomass has proven to promote hemicellulose acetylation, and enhanced the mechanical properties and hydrophobicity of hemicellulose-based films. This section provided the basis for selecting wheat straw pre-treatment conditions used for tailor-making hemicellulose properties for food packaging material development.

Authors: Lindleen Mugwagwa, Annie Chimphango

Summary of authors' contributions

Lindleen Mugwagwa carried out the planning and experimental work for the papers in this chapter,. Analysis and interpretation of the results was a combined contribution of both authors. Lindleen Mugwagwa did 80% of the work presented in this chapter while 20% was Annie Chimphango's contribution.

5.1. Paper Title: "Optimising wheat straw alkali-organosolv pre-treatment to enhance hemicellulose modification and compatibility with reinforcing fillers "

Published in the journal of Biological macromolecules (impact factor 5.162)

5.2. Abstract

Hemicellulose acetylation, filler compatibility and film hydrophobicity were enhanced by optimising alkali-catalysed organosolv treatment conditions for wheat straw before hemicellulose extraction. Two optimum (OPT) conditions were established by varying organosolv components' concentrations [EC (50–80 %) and NaOH (1–13%)], and time (2–6 h), in a Box-Behnken experimental design. The OPT1 (1% NaOH/50% EC/6 h) that enhanced acetylation and filler compatibility, and OPT2 (13% NaOH/80% EC/5 h) that enhanced hydrophobicity by minimising DS, UAC and arabinose/xylose ratio. The films made from OPT2 hemicellulose had higher WCA (68.1^{o}) than OPT1 hemicellulose (39.90^{o}). However, OPT1 hemicellulose had a higher DS (1.7 vs 0.4), and their acetylated nanocellulose reinforced films had higher tensile strength (10.59 vs 4.87 MPa) and Young's modulus (590.15 vs 323.64 MPa) than OPT2 hemicellulose. Therefore, hemicellulose acetylation, hydrophobicity and filler interaction can be engineered by manipulating the organosolv treatment conditions.

Keywords*:* Alkali-catalysed organosolv pre-treatment; Wheat straw; Hemicellulose; Acetylation; Biofilms; Box-Behnken design

5.3. Introduction

There has been an increased interest in the utilisation of biomass-derived films as a replacement for non-renewable, non-biodegradable petroleum-based films (Ashter, 2016). Consequently, the use of lignocellulosic biomass as a viable raw material for cheap, renewable and biodegradable biofilms has increased (Hu *et al.*, 2016; Liu *et al.*, 2018). Among the lignocellulosic cell wall matrix components; that is, cellulose, lignin and hemicellulose, the latter is the most underutilised because of its hydrophilic nature and poor mechanical properties (Akkus *et al.*, 2018). However, the use of cellulose and lignin-based biofilms cannot cope with the increased demand for biopolymers. As a result, this has led to a rising research interest in the utilisation of hemicellulose to widen the raw material options for film production. Hemicellulose has excellent film-forming properties and it can be engineered for different applications (Chen *et al.*, 2016; Farhat *et al.*, 2016). Agricultural residue such as wheat straw is an abundant raw material for hemicellulose as it contains 20-40% hemicellulose, particularly, arabino-4-O-methylglucuronoxylan (Borand and Karaosmanoğlu, 2018). The utilisation of wheat straw can be broadened by fractionating it to recover biopolymers such as hemicellulose, in addition to the straw's traditional uses as animal feed, organic manure and a bioenergy source (Batidzirai, Junginger, *et al.*, 2016).

The hydrophilicity of arabino-4-O-methylglucuronoxylan is attributed to the presence of arabinose and uronic acids (glucuronic acid) side chains on its structure (Sun and Tomkinson, 2003; Sternemalm *et al.*, 2008). Hydroxyl groups on these side chains form hydrogen bonds with water, thereby compromising the mechanical and barrier properties of the hemicellulose-based films, (Egüés *et al.*, 2013; Gomes *et al.*, 2015; Akkus *et al.*, 2018). Acetylation, a process that involves replacing the hydrogen in hydroxyl groups of hemicellulose with acetyl groups, is a promising strategy for the enhancement of mechanical and moisture barrier properties of hemicellulose-based films (Pawar *et al.*, 2013; Egüés *et al.*, 2014). However, acetylation does not modify all the available hydroxyl groups in the hemicellulose, hence, hemicellulose will still have a high affinity for moisture regardless of achieving a high degree of acetylation (DS 1.8) (Gordobil *et al.*, 2014). There is potential to increase the effectiveness of the acetylation process by reducing the side chains in hemicellulose before acetylating.

The quantity of side chains in hemicellulose depends significantly on pre-treatment and extraction conditions (Li and Pan, 2018). Pre-treatments have included enzymatic cleaving of side chains to diversify hemicellulose functionalities (Heikkinen *et al.*, 2013; Kochumalayil and Berglund, 2014). However, enzymatic treatment is costly, especially for large scale modification (Chapman *et al.*, 2018). Alternatively, fractionation conditions can be manipulated to recover hemicellulose with an optimum composition for effective acetylation, which would, in turn,

enhance hemicellulose hydrophobicity. However, research has focused more on manipulating acetylation process conditions to increase DS (Xu *et al.*, 2008; Lin *et al.*, 2010) than manipulating the hemicellulose pre-treatment and extraction conditions.

Alkali-catalysed organosolv treatment is a promising method for treating biomass prior to hemicellulose extraction. The organosolv method utilises low-boiling-point organic solvents such as ethanol, methanol, butanol and acetone coupled with alkaline catalysts such as sodium hydroxide and potassium hydroxide for biomass fractionation (Mesa *et al.*, 2011; Guragain *et al.*, 2016). The alkali-catalysed organosolv pre-treatment selectively cleaves the linkages between hemicellulose and lignin in the biomass minimising the degradation of hemicellulose into the undesirable monomeric form (Liu *et al.*, 2016). Depending on severity, alkali-based organosolv has the potential to remove the side chains from hemicellulose that contribute to hemicellulose affinity for water. Alkali-based organosolv cleaves uronic acid and arabinose side chains from biomass and breaks covalent bonds (α ester linkages) between hemicellulose and lignin (Peng, Peng, Xu and R. Sun, 2012; Geng *et al.*, 2018). Research carried out so far does not demonstrate the potential of the alkali-catalysed organosolv pre-treatment method for enhancing hemicellulose hydrophobicity in films. Instead, alkali-based organosolv is applied to improve enzymatic digestibility of the biomass for ethanol production or to facilitate bleaching of celluloses in papermaking (Shatalov and Pereira, 2004; Mesa *et al.*, 2011; Diner and Fan, 2012).

Optimisation of alkaline catalysed organosolv conditions for the removal of side chains can enhance the effectiveness of the acetylation process and crystallinity of hemicellulose, and subsequently, the hydrophobicity of hemicellulose-based films. Apart from hydrophilicity, hemicellulose-based films have weak mechanical properties and, thus, require fillers as reinforcement to improve their structural properties (Pereira *et al.*, 2017; Huang *et al.*, 2018). Reinforcement of AH with ACNC improves the Young's modulus and tensile strengths of AH-based films (Egüés *et al.*, 2014; Gordobil *et al.*, 2014). However, the effectiveness of ACNC as a reinforcement material in a hemicellulose matrix depends mainly on matrix-filler compatibility (Gordobil *et al.*, 2014). The effects of alkali-based organosolv on the interaction of AH and ACNC in films are not clear. This study investigated the potential to engineer alkali-based organosolv pre-treatment conditions of wheat straw in order to enhance the mechanical and functional performance of hemicellulose-based films.

Therefore, the objective of the optimisation of the alkali-based organosolv treatment of wheat straw was two-fold, namely to improve the acetylation process for increased hydrophobicity and filler-matrix interaction in films. The optimisation of the organosolv treatment conditions was based on the hemicellulose composition in terms of UAC, arabinose/xylose ratio and lignin contents, and

functional properties in terms of hydrophobicity and mechanical performance in films (tensile and Young's modulus), which was done using a Box-Behnken experimental design.

5.4. Materials and methods

5.4.1. Chemicals

The NaOH, ethanol, sulphuric acid, HCl, and phenolphthalein indicator were sourced from Kimix Chemical & Lab Supplies, Cape Town, South Africa. Carbazole, glucuronic acid, acetic anhydride, acetic acid, dimethyl sulfoxide (DMSO), and sugar standards (glucose, arabinose and xylose) were purchased from Sigma Aldrich, Germany. All the chemicals were of analytical grade. Freeze dried nanocellulose nanocrystals (made using sulphuric acid as the chemical reagent) were purchased from the University of Maine, Orono, USA.

5.4.2. Sample preparation

Wheat straw was supplied by local wheat farmers in Cape Town, South Africa. The wheat straw was milled to 80-mesh size with a Retch ZM200 mill and, subsequently, sieved using a horizontal sieve shaker based on the British Standard Sieving Test Procedure, BS 410, to remove fines. The wheat straw was packed into sacks and stored in a dry place until the experimental work commenced.

5.4.3. Alkali-based organosolv treatment of wheat straw

Wheat straw (25 g) was treated with 250 mL ethanol/NaOH mixture consisting of 50–80% ethanol and 1–13% NaOH, giving a solid/liquid ratio of 1:10 (w/v). The alkali catalysed organosolv treatment was carried out in Schott bottles incubated at 75 ^{o}C in a shaking water bath for 2–6 h. After the treatment, the solid residue (delignified wheat straw) was washed five times with 500 mL of water to remove any traces of the black liquor before oven-drying at 40 ^{o}C for three days to a moisture content < 10%.

5.4.4. Hemicellulose extraction

Hemicellulose was extracted from the alkali-organosolv treated wheat straw according to a method by Shimokawa *et al*. (2015) and Egüés *et al*. (2014). Wheat straw (10 g) was treated with 100 mL of 10% (w/v) NaOH, giving a solid/liquid ratio of 1:10 (w/v), for 24 h at 25 ^{o}C in a shaking water bath. The reaction mixture was vacuum-filtered using 45-µm filter paper. The hemicellulose was recovered by precipitation, which was achieved by reducing the pH of the supernatant to pH 5.5 with 10% HCl. Subsequently, the precipitates were dialysed using a cellulose dialysis tube membrane with a molecular weight cut off of 14 000 (Sigma Aldrich) placed in distilled water for three days to remove the salts. The hemicellulose was then recovered by centrifugation (8000 rpm for 30 min) followed by oven-drying for three days at 40 ^{o}C to a moisture content of less than 10%.

5.4.5. Hemicellulose acetylation

The acetylation of the hemicellulose was done according to Belmokaddem *et al*. (2011) but with sulphuric acid used as a catalyst instead of methanesulfonic acid use in the original method. Hemicellulose (1 g) was added to 2.5 mL of 99% acetic acid in a Schott bottle and incubated in a water bath set at 50 ^{o}C for 5 min. The hemicellulose mixture was then cooled in an ice-bath to 25 ^{o}C before adding 6.6 mL of 99% acetic anhydride and 200 µL of 98% sulphuric acid as a catalyst (the process was performed in a fume hood). The acetylation reaction was allowed to take place for 1 h in a shaking water bath at 50 ^{o}C. Thereafter, the AH was precipitated with 150 mL of 95% ethanol. The residual acetic anhydride was removed from the AH by washing four times with 100 mL of 95% ethanol before oven-drying for three days at 40 ^{o}C. The AH was stored in tightly closed plastic tubes before analysis.

5.4.6. Nanocellulose acetylation

Nanocellulose acetylation was carried out according to Sahlin, (2015) with modifications (temperature of 25 ^{o}C and 1 µl sulphuric acid was used in this study instead of 40 ^{o}C and 200 µl respectively used in the original method). Approximately, 300 mg of nanocellulose was thoroughly mixed with 3 mL of 99% acetic acid for 1 min in a Schott bottle. A mixture of 99% acetic anhydride (1 mL) and 1 µl of 98% sulphuric acid were then added to the nanocellulose slurry, which was left in a water bath at 25 ^{o}C for 3.5 h to allow acetylation to take place. The ACNC was recovered by precipitation with 150 mL of water followed by washing the ACNC three times with 150 mL water. The ACNC was oven-dried at 40 ^{o}C for 24 h to a moisture content of less than 10%.

5.4.7. Hemicellulose films preparation

The AH (500 mg) was dissolved in (20 mL) dimethyl sulfoxide by heating the mixture at 50 ^{o}C while stirring at 800 rpm for 6 h using a Stuart 152 magnetic stirrer hot plate. Between 10%–50% w/w ACNC (based on the total solids weight), was added to the AH/dimethyl sulfoxide solution to produce the hemicellulose films with ACNC reinforcement. The hemicellulose-based films were formed by pouring the 20 mL mixture in 80 mm glass Petri dishes and drying these in an oven at 50 ^{o}C for two days, which resulted in producing films with a thickness of 86 µm ± 4. The film thickness was measured using a model 456CFSS elcometer.

5.4.8. Analysis and characterisation of hemicellulose, nanocellulose and hemicellulose-based films

The hemicellulose yield was calculated based on Equation 16 below.

$$Hemicellulose\ yield\ (\%) = \frac{Hemicellulose\ recovered}{Initial\ hemicellulose\ content\ of\ biomass} \times 100\% \qquad (16)$$

The hemicellulose sugar and lignin contents were determined according to the NREL/TP-510-42618 procedure (Sluiter *et al.*, 2012). The arabinose/xylose ratio, xylose, lignin, glucose and UAC contents of the hemicellulose were determined by a two-stage acid hydrolysis method described by Brienzo *et al*. (2009). Briefly, 300 mg of hemicellulose was placed in a test tube and hydrolysed with 1.5 mL of sulphuric acid at 45 ^{o}C for 7 min in a water bath. Thereafter, the hydrolysate was diluted to an acid concentration of 3% by the addition of 45 mL of water. Further hydrolysis was facilitated by autoclaving the diluted hydrolysate at 121 ^{o}C for 30 min. A 10 mL liquid fraction was collected for analysis after removal of solid residues by vacuum-filtration using glass filters.

The pH of the hydrolysate was adjusted to between pH 3 and pH 7 with 7 M KOH, and the neutralised hydrolysate was subsequently, filtered using 0.22-µm nylon filters (supplied by Lasec, South Africa) before HPLC sugar analysis. The HPLC equipment used was equipped with a BioRad Aminex HPX-87 column, a Cation-H Micro-Guard Cartridge and Shodex R I101a refractive index detector. The HPLC column was set at 65 ^{o}C and the mobile phase, 5 mM sulphuric acid, was maintained at a flow rate of 0.6 mL/min. Sugar standards (arabinose, xylose and glucose) were used to develop calibration curves for quantification of the respective sugars.

The lignin content of the hemicellulose was determined by measuring the absorbance of the hemicellulose hydrolysate at a wavelength of 240 nm using a Varian Cary 50 Bio UV Visible (UV-Vis) spectrophotometer.

The UAC of the hemicellulose was determined according to Brienzo *et al*. (2009) and Chimphango, (2010). The method involved mixing 0.4 mL of the acidic hydrolysate with 2.4 mL of 98% sulphuric acid, then allowing it to cool to 25 ^{o}C before adding 0.1 mL (0.1% w/v) carbazole in 95% ethanol. The mixture was allowed to react at 100 ^{o}C for 20 min in a water bath followed by cooling in an ice-bath to 25 ^{o}C. Uronic acids were determined by measuring the absorbance of the mixture at 525 nm using Varian Cary 50 Bio UV-Vis spectrophotometer. Standard curves of glucuronic acid were developed and used for uronic acids quantification.

The AH and ACNC were analysed for the acetylation DS according to Gordobil *et al*. (2014). However, 0.1 M solutions of NaOH and HCl were used instead of 0.5 M used in the original method. Acetylated and unmodified hemicellulose and nanocellulose samples were reacted with 10 mL of 75% ethanol in a shaking water bath at 50 ^{o}C for 30 min. The mixtures were cooled to 25 ^{o}C before addition of 0.1 M NaOH. Titration was carried out with 0.1 M HCl to the phenolphthalein endpoint. Further titration of the mixtures was carried out after 2 h to neutralise any excess alkali released by the samples. The acetyl content of the hemicellulose and nanocellulose was determined using Equation 17.

$$Acetyl\ content\ (\%) = \frac{(V_a - V_b) \times N_{hcl} \times M_{acetyl}}{M_s} \times 100 \quad (17)$$

where:

V_a = Titer volume for the unmodified samples in litres

V_b = Titer volume for the acetylated sample in litres

N_{hcl} = Molarity of the HCl used

M_{acetyl} = Molecular weight of the acetyl group (43 g/mol)

M_s = Weight of the AH or ACNC in grams used in the analysis

The DS of the AH and ACNC was calculated taking into consideration the number of acetyl groups introduced onto the hemicellulose or nanocellulose structure by acetylation. The DS was obtained as follows (Equation 18):

$$Degree\ of\ substitution = \frac{Acetyl\ content \times 162}{[(M_{acetyl} \times 100) - (M_{acetyl} - 1)] \times Acetyl\ content} \times 100 \quad (18)$$

where: M_{acetyl} = molecular weight of the acetyl group- 43 g/mol

The WCA of the AH films was measured with a stereomicroscope (Nikon SMZ-2T, Japan) equipped with a camera. Approximately 1µL droplets of deionised water were placed on the film surface and their images captured by the camera. The water droplet-film contact angle was then measured from the images using Scion Image software.

The mechanical properties, that is, tensile strength, Young's modulus and percent elongation of the films were analysed with an LRX Instron tensile tester by LLOYD instruments. The equipment was set at a crosshead speed of 50 mm/min and gauge length of 27.3 cm. Hemicellulose film strips cut to a length of 6 cm and width of 1 cm were used for the analysis.

5.4.9. Assessing the effect of alkali catalysed organosolv treatment on hemicellulose composition, acetylation and hydrophobicity

The influence of the alkali-catalysed organosolv treatment of wheat straw on hemicellulose composition, acetylation of hemicellulose and hemicellulose-based films hydrophobicity was investigated using a Box-Behnken experimental design using Statistica 7, version 13.2. The experimental design had three factors varied at three levels; EC (50, 65 and 80%), NaOH (1, 7 and 13%) and time (2, 4 and 6 h), resulting in 15 experimental runs which had 3 centre points as shown in Table 14. The correlation of hemicellulose composition to the DS as well as to the hydrophobicity of the films produced, were studied using the correlation matrices (Statistica 7 version 13.2). Derringer's desired function methodology was used to determine optimal alkali-

organosolv treatment conditions. Two optimum conditions for the alkali-based organosolv treatment were obtained by multi-objective optimisation of the response variables based on Derringer's desired function methodology. The first optimisation procedure aimed at finding the optimal organosolv treatment conditions (OPT1) that enhance the acetylation process with the overall goal of increasing film hydrophobicity. Therefore, the objective for OPT1 was to maximise both the DS and WCA of the hemicellulose films. The second optimisation procedure (OPT2) focused on improving films hydrophobicity by manipulating hemicellulose composition and minimising the DS required. The objective of OPT2 was to minimise uronic acids, arabinose/xylose ratio and DS and to maximise hemicellulose water content angle and lignin content of the hemicellulose. The Tukey's test was carried out with Statistica 7 version 13.2 software, to compare the means of mechanical properties.

5.5. Results and discussions

5.5.1. Effect of wheat straw organosolv treatment on hemicellulose yield and composition

Hemicellulose yield from wheat straw was significantly affected ($p < 0.05$) by EC, NaOH concentration, treatment time and the interaction between the NaOH concentration and extraction time (Figure 27). The highest hemicellulose yields > 39% were obtained from wheat straw that was delignified with 1% NaOH followed by organosolv treatment with 7% NaOH (up to 21.58%). The least hemicellulose recovery (< 12%) was obtained with organosolv treatment of 13% NaOH concentration, which occurred irrespective of EC and extraction time (Table 14).

The decline in hemicellulose yield with the increase in NaOH concentration from 1% to 13% (Figure 28a and b) observed in this study was due to peeling reactions and alkaline-based hydrolysis of the hemicellulose with an increase in severity of organosolv treatment conditions (Guragain *et al.*, 2016). Chen *et al*. (2013) highlighted that hemicellulose and other carbohydrates degradation was more pronounced at NaOH loading exceeding 8%, but the findings in this study show that the rate of hemicellulose degradation was prominent as NaOH concentration increased from 1% to 8% (Figure 28b).

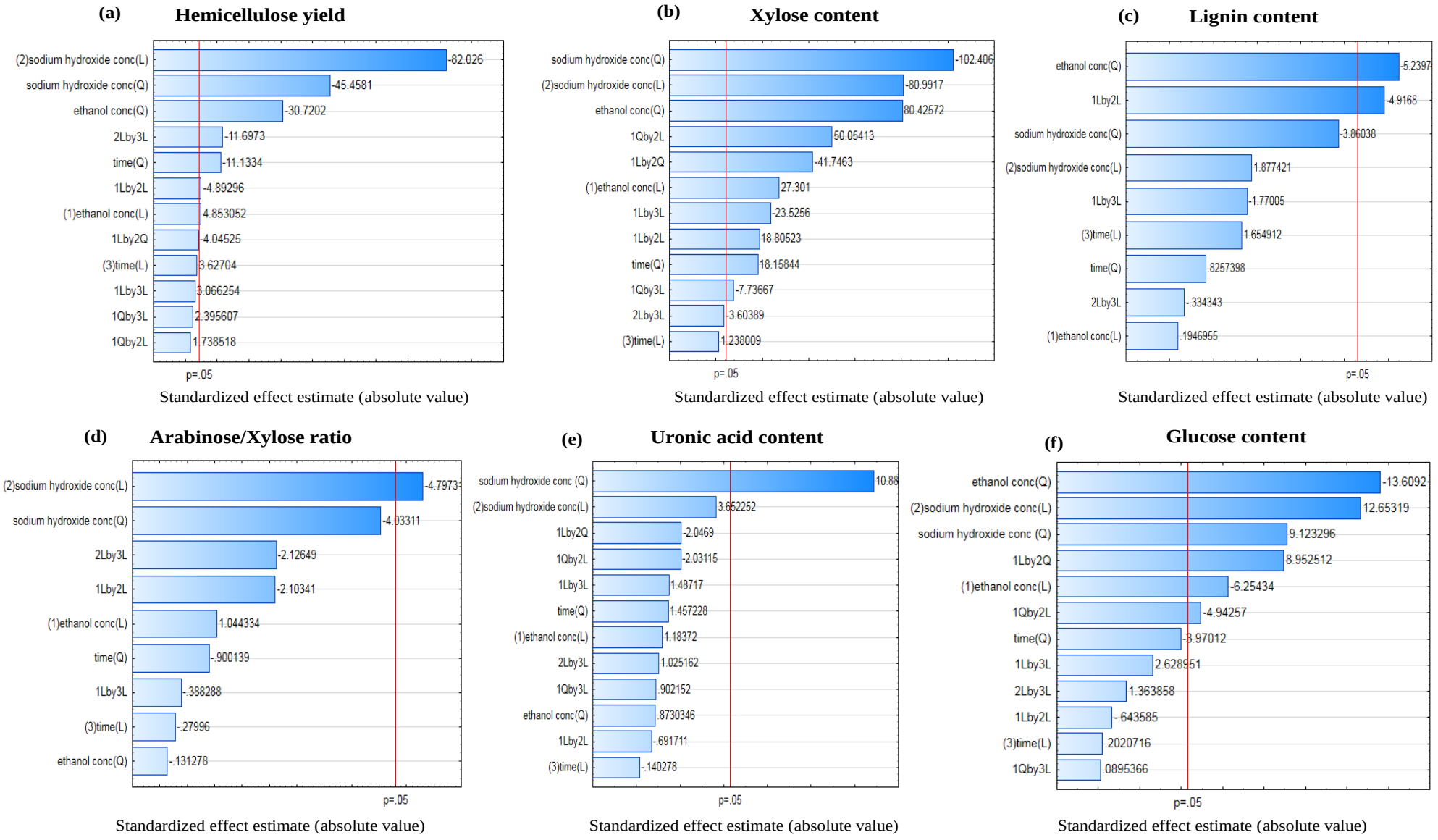


Figure 27: Pareto charts showing the standardised effects of organosolv treatment conditions on the hemicellulose composition

An increase in EC from 50% to 80% at lower NaOH concentration (> 2%) during the pre-treatment had a shielding effect to chemical attack of the biomass thus, preventing hemicellulose hydrolysis in the alkali medium, thereby promoting higher extraction yields from the delignified wheat straw (Figure 27). The results are in agreement with the results of Shatalov and Pereira, (2002) in which an increase in hemicellulose yield from 8% - 12.13% was observed when EC was increased from 20 - 60%. Thus, organosolv treatment of wheat straw with NaOH concentration of less than 2% and EC of 80% could be the most effective conditions in realising the highest hemicellulose yield (Figure 28a).

The results evidently show that there is an optimum NaOH concentration that can be used during organosolv treatment beyond which substantial losses in the hemicellulose yield occur. Therefore, in order to maximise hemicellulose yield, it is critical to optimise the NaOH catalysed organosolv treatment conditions focusing on identifying the maximum NaOH concentration and minimum EC for a given extraction time. This will be essential to fulfill the goal of obtaining sufficient quantities of hemicellulose for biofilms production.

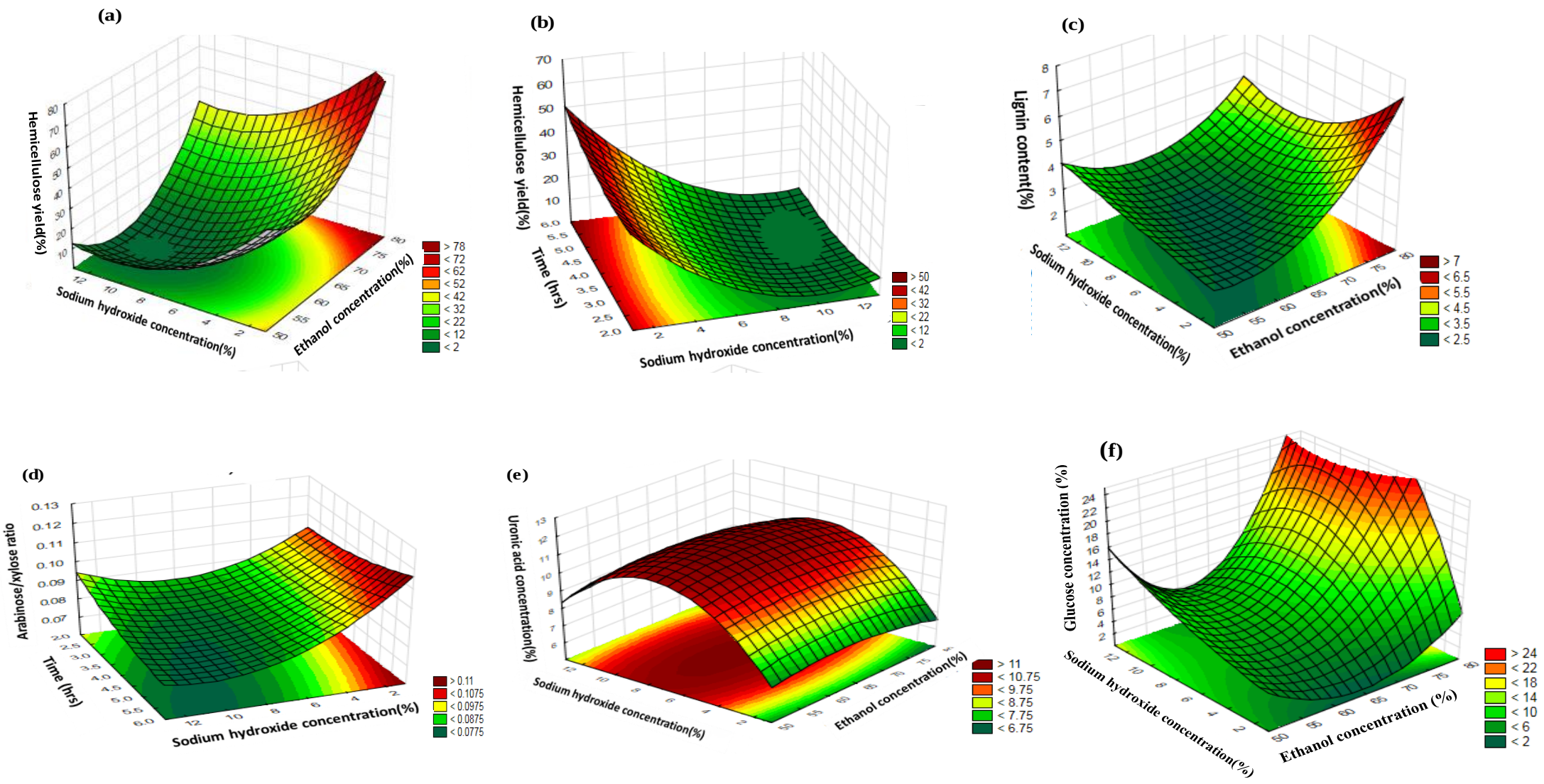


Figure 28: Effects of organosolv treatment conditions on hemicellulose composition

The alkali-based organosolv treatment conditions yielded hemicellulose from wheat straw with varying sugar composition as evidenced by the distinct variation in xylose, arabinose, glucose, lignin and uronic acids contents (Table 14) at different treatment conditions. The major contributors to the hemicellulose yield were the xylose sugars which contributed 69.06 - 82.55% and a small fraction of side chains (arabinose and uronic acids, lignin and glucose) making up 17.45 - 30.94% of the hemicellulose (Table 14).

The arabinose/xylose ratio and UAC were significantly influenced by only one variable, which is NaOH concentration (Table 14, Figure 28d and e), whereas glucose content was affected by ethanol and NaOH concentration and their interaction (Table 14, Figure 28f). The xylose content of the hemicellulose was significantly ($p < 0.05$) affected by all the organosolv treatment variables and their interactions at the 95% confidence level (Figure 28b). From Table 14 it is apparent that lignin content was the only response variable which was not significantly ($p > 0.05$) affected by any of the organosolv treatment variables. NaOH concentration had the most influence on arabinose/xylose ratio, UAC and xylose content of the hemicellulose whereas EC was the most influential factor on glucose and hemicellulose content (Figure 28).

As the total side groups increased (from 17.45% to 30.94%), especially the glucose (from 1.45% to 14.33%) and the UAC (from 6.37% to 12.38%), in the hemicellulose extracts, the xylose content (from 82.55% to 69.06%) diminished (Figure 29), and consequently the hemicellulose yield (56.01% to 3.2%) decreased (Figure 30). Similarities in trends for arabinose/xylose ratio (Figure 28a) and the hemicellulose yield (Figure 28d) with variation in NaOH concentration and time proves that the removal of the arabinose side chains had a negative impact on the hemicellulose yield. An accumulation in glucose in the hemicellulose extract with higher NaOH concentrations (Figure 28f) was realised, and this could have been due to the harsh organosolv treatment conditions modifying cellulose in the wheat straw making it more susceptible to hydrolysis with NaOH to glucose during the hemicellulose extraction process (Knill and Kennedy, 2003). In addition, the hemicellulose itself could have also undergone the same degradation reaction at the severe organosolv treatment conditions, thereby releasing glucose and losing some of the xylose (Figure 30) in the process (Peredo *et al.*, 2016). Green and Sanyer, (1982) also made the same conclusion when they observed that high alkali concentrations during pulping led to the depolymerisation of cellulose from wood. The inverse relationship observed between the hemicellulose extract's glucose content and xylose content (Figure 29) depicts that the harsh conditions do not only reduce the yield of hemicellulose but also increase co-extraction of glucose.

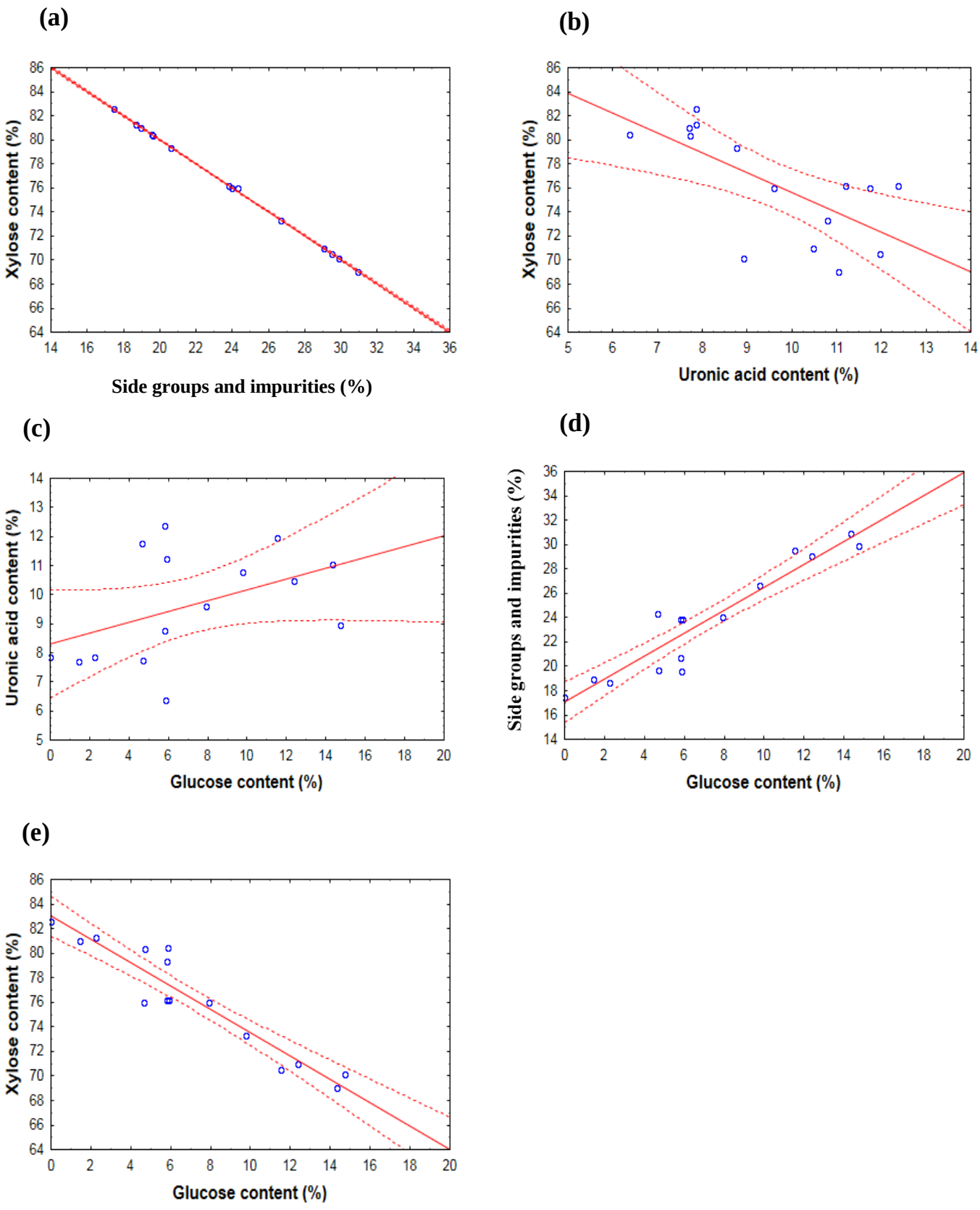


Figure 29: Correlations of xylose, side groups in the hemicellulose extracts. The correlation coefficient (r) for (a) = -1 (b) = -0.68 (c) = +0.45 (d) = +0.94 and (e) = -0.95

On the other hand, UAC build up in the hemicellulose was observed with an increase in NaOH concentration from 1% to approximately 7%, while at higher concentration the UAC declined. The increase in organosolv treatment medium alkalinity as NaOH concentration increased inhibited the cleaving of uronic acids from the hemicellulose in the wheat straw as uronic acids require acidic solutions for their removal from biomass (Li *et al.*, 2007; Voragen *et al.*, 2009). Alkaline organosolv treatment of the wheat straw promotes removal of acetyl groups from the biomass. Accumulation of the acetyl groups in the organosolv treatment medium with lower alkalinity can significantly reduce the pH of the organosolv treatment medium. Therefore, organosolv treatment at lower NaOH concentration could have been affected by the reduction in pH due to neutralisation of the organosolv treatment medium by the acetyl groups released during the organosolv treatment process.

The lower the pH of the organosolv treatment solution the higher the removal of uronic acids from the hemicellulose in wheat straw due to increased dissolution of the uronic acids in acidic medium (Faravash and Ashtiani, 2007; Kermani *et al.*, 2015). A decrease in UAC was observed as NaOH increased from 7% to 13% and this could have been due to the conversion the methylglucuronic acid to 4-deoxy-β-ι-threo-hex-4 enopyranosyluronic acid (hexenuronic acid) by β elimination of methanol (Shatalov and Pereira, 2002). Alkali-based organosolv treatment has demonstrated the capability to modify the hemicellulose structure in wheat straw before the hemicellulose has been extracted. Generally, the hemicellulose structure had its side groups removed with an increase in organosolv treatment severity and, this in turn, reduced the hemicellulose yield. Although the yield was compromised, the removal of these side groups could be a move towards reducing hemicellulose affinity for moisture.

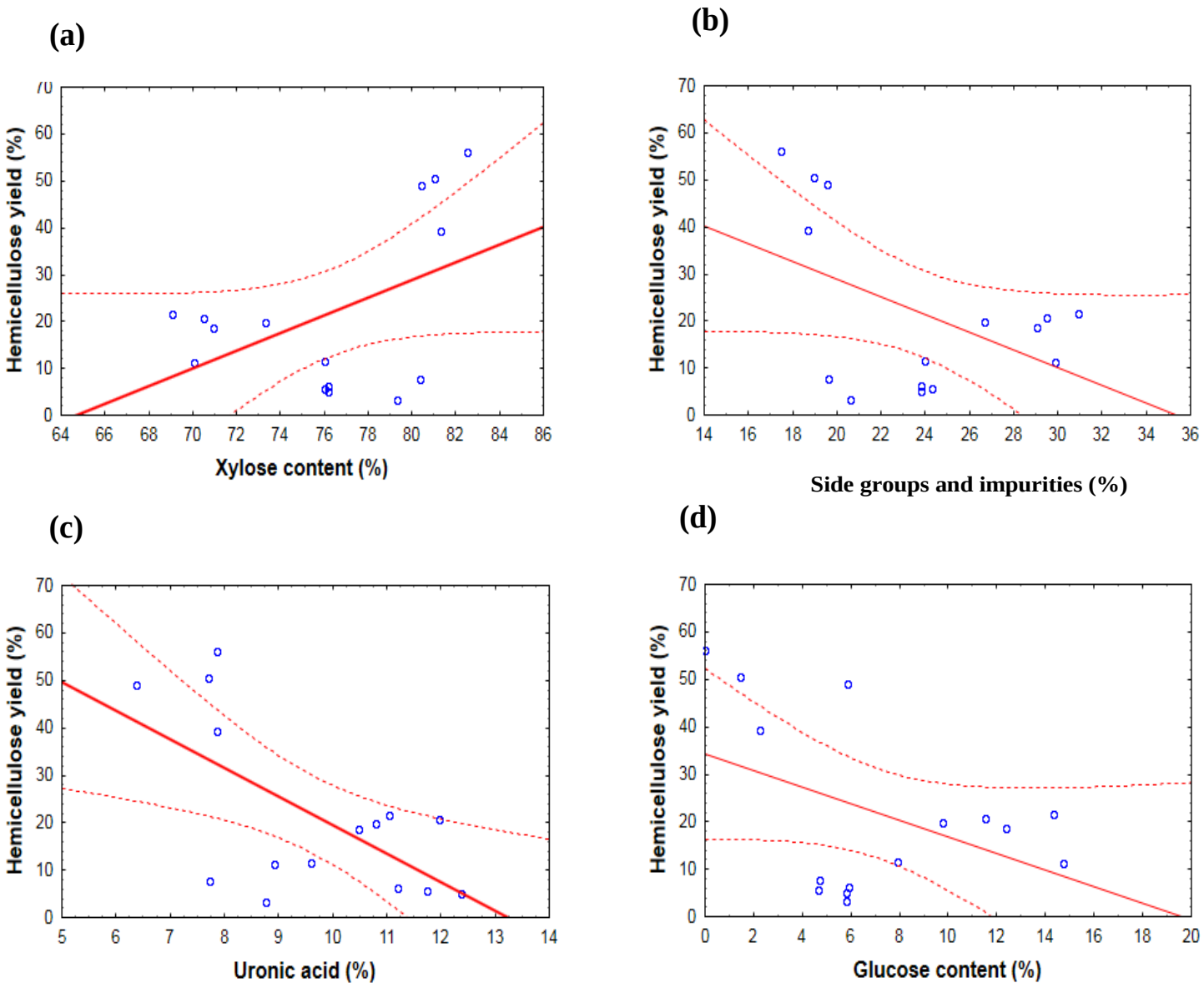


Figure 30: Contribution of hemicellulose components to the hemicellulose yield. The correlation coefficient (r) for (a) = +0.47 (b) = -0.48 (c) = -0.63 and (d) = -0.44

5.5.2. Effect of organosolv treatment on hemicellulose acetylation

Hemicellulose acetylation reflected based on the DS was significantly affected ($P < 0.05$) by varying NaOH and EC in alkaline catalysed organosolv pre-treatment as shown in Table 14. In the table, the DS of the AH ranges from 0.11 to 1.7, which correspond to different ethanol and NaOH combinations in the pre-treating solvent. The results suggest that it is possible to engineer hemicellulose DS by controlling the extent of wheat straw alkali-organosolv treatment before hemicellulose extraction and acetylation. Among the process conditions, EC had the most influence on the acetylation process followed by the interaction of NaOH and EC, then NaOH, while organosolv treatment time had the least effect (Figure 31a).

***Table 14**: Hemicellulose composition, acetylation and film water contact angle at different organosolv treatment conditions*

Organosolv treatment			**Hemicellulose composition**							**Acetylation**	**Film hydrophobicity**
EC (%)	NaOH (%)	Time (h)	Hemicellulose yield (%)	Xylose content (%)	Side groups (%)	Glucose content (%)	Arabinose/Xylose ratio	Lignin content (%)	Uronic acid content (%)	DS	WCA (°)
80	1	4	49.9	80.41	19.59	5.88	0.091	2.51	6.37	1.7	31.34
80	1	4	56.01	82.55	17.45	1.75	0.116	4.28	7.86	0.11	25.82
50	13	4	11.18	70.09	29.91	14.74	0.089	4.08	8.94	1.07	57.58
80	13	4	11.6	76.00	24.00	7.94	0.085	3.35	9.61	0.55	76.26
50	7	2	20.66	70.51	29.49	11.55	0.085	2.46	11.97	0.49	19.88
80	7	2	18.5	70.96	29.04	12.38	0.087	2.46	10.48	0.69	39.67
50	7	6	19.67	73.33	26.67	9.76	0.084	3.86	10.79	0.64	52.83
80	7	6	21.58	69.06	30.94	14.33	0.081	2.96	11.05	0.55	35.53
65	1	2	39.43	81.32	18.68	2.26	0.105	2.71	7.86	1.39	36.23
65	13	2	7.67	80.36	19.64	4.69	0.090	3.15	7.74	0.45	64.22
65	1	6	50.5	81.04	18.96	1.45	0.121	2.44	7.7	1.42	35.64
65	13	6	3.20	79.36	20.64	5.83	0.076	2.71	8.77	0.21	42.57
65	7	4	4.91	76.16	23.84	5.83	0.074	2.06	12.38	0.66	44.20
65	7	4	6.22	76.18	23.82	5.93	0.088	2.53	11.21	0.74	43.00
65	7	4	5.76	76.00	24.32	4.65	0.079	2.46	11.75	0.6	40.65
Factor			**P values (3 decimal places)**								
(1)EC			0.002	0.000	0.002	0.009	0.644	0.066	0.480	0.011	0.121
(2)NaOH			0.000	0.000	0.001	0.008	0.0484	0.100	0.015	0.019	0.003
(3) Time			0.014	0.006	0.056	0.112	0.692	0.199	0.483	0.638	0.047
1*2			0.065	0.000	0.006	0.028	0.170	0.088	0.265	0.011	0.025
1*3			0.117	0.003	0.028	0.224	0.735	0.109	0.398	0.267	0.010
2*3			0.007	0.069	0.347	0.306	0.167	0.770	0.413	0.195	0.0280

EC: Ethanol concentration, NaOH: Sodium hydroxide concentration, DS: Acetylation degree of substitution, WCA: Water contact angle

Ethanol solutions at high pH have been reported to break lignin bonds in biomass and hemicellulose to lignin bonds, thereby facilitating removal of lignin from biomass leaving hemicellulose intact in the biomass (Borand and Karaosmanoğlu, 2018). Hemicellulose, after the pre-treatment, has lower binding lignin, hence, it has more sites for modification by acetylation than hemicellulose extracted from unpretreated wheat straw. Therefore, for effective hemicellulose acetylation, optimisation of EC and NaOH concentration used for organosolv treatment is important, as these have proven to diversify hemicellulose composition and structural features, which influence acetylation (Table 14). For instance, it is evident from the results that varying organosolv treatment conditions varied the hemicellulose composition with respect to hemicellulose arabinose and uronic acid side groups (17.45–30.94%), xylose content (69.09–82.55%) and lignin content (2.06–4.28%) (Table 14), which in turn, varied the DS (Figure 32). Therefore, acetylation of hemicellulose can be manipulated by controlling the hemicellulose composition and side group content.

Notably, the alkali-organosolv treatment conditions that spared arabinose side chains in hemicellulose, that is a decrease in NaOH concentration from 13% to 1% in the pre-treatment solvent, boosted the acetylation process (Figure 31b, c, d and e). The presence of the arabinose side chains contributed to the number of free hydroxyl groups available for substitution with acetyl groups during acetylation, thereby enhancing the acetylation process (Gordobil *et al.*, 2014). Furthermore, this phenomenon was supported by a positive correlation between the arabinose/xylose ratio and DS highlighted in Figure 32a. Therefore, alkali-organosolv treatment conditions that minimise hydrolysis of arabinose side chains would favour the acetylation process.

The hemicellulose DS was higher for hemicellulose samples that were recovered from wheat straw after the alkali-catalysed organosolv pre-treatment was performed with lower NaOH concentration and EC (Figure 31a and b). The lower EC and high pH facilitated removal of lignin (Table 14), which otherwise hindered the acetylation process (Figure 32c). Peredo *et al*., (2016) provided supporting evidence when they achieved higher DS in cellulose with lower lignin content when compared to cellulose with higher lignin content (Peredo *et al*., 2016). Residual lignin in hemicellulose reduces the surface area in hemicellulose available for acetylation, due to the impervious nature of lignin to chemical attack (Minato *et al.*, 2007). Hence, the removal of lignin by breaking the lignin-hemicellulose covalent bonds during the alkali-organosolv treatment can increase the sites for acetylation by freeing hydroxyl groups in hemicellulose (Egüés *et al.*, 2014; Peredo *et al.*, 2016). Although statistically the organosolv treatment conditions had no significant effect ($p > 0.05$) on the lignin content of the hemicellulose (Table 14), the variation in the organosolv treatment conditions varied the hemicellulose lignin content to the extent that it impacted acetylation (Figure 32c).

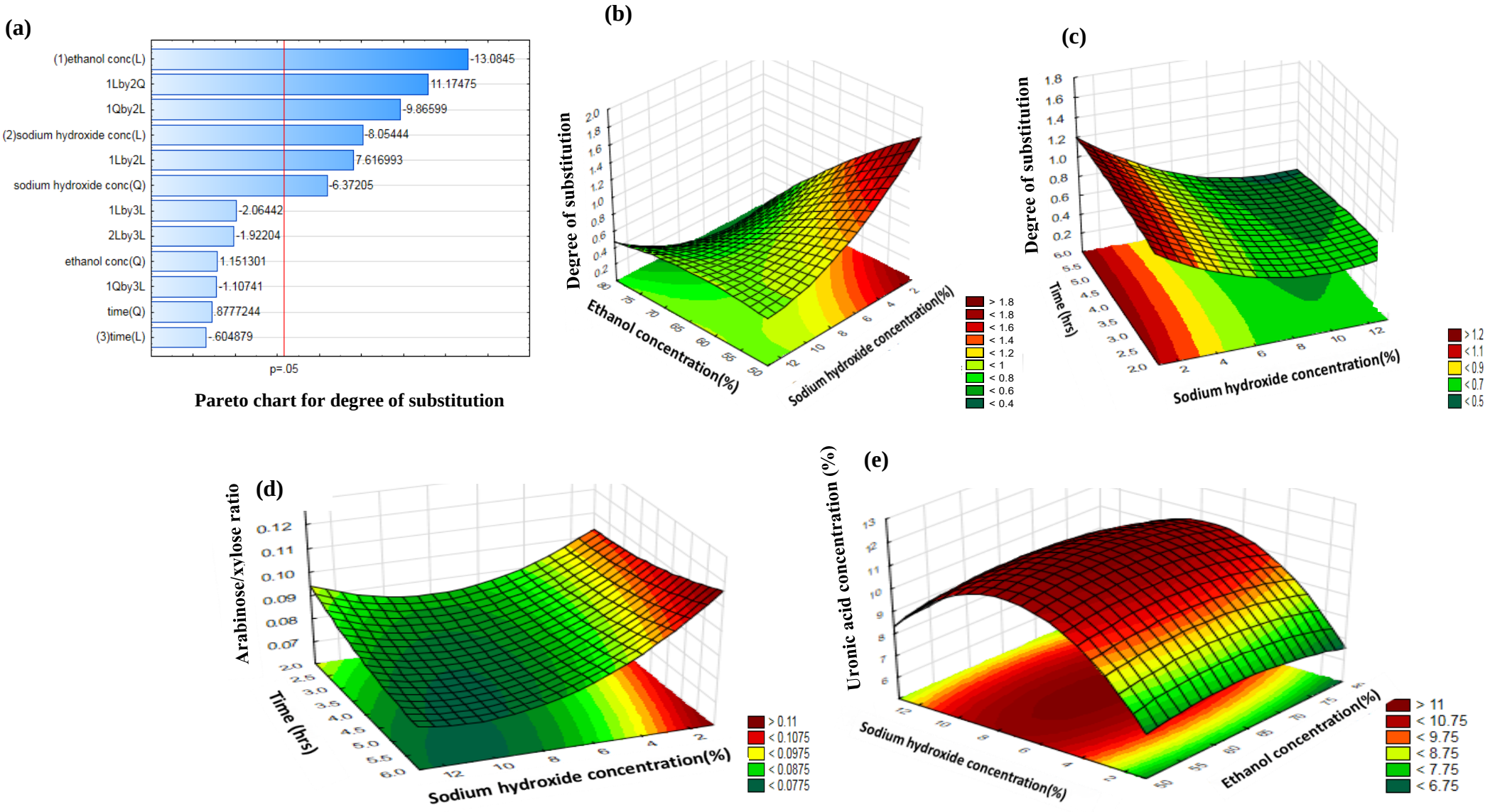


Figure 31. Effects of organosolv treatment conditions on acetylation degree of substitution, hemicellulose arabinose/xylose ratio and uronic acids content

The alkali-organosolv treatment conditions that favoured high UAC in the hemicellulose inhibited the acetylation process (Figure 32b). There was a decrease in acetylation DS with an increase in UAC most likely because of the strong ester bonds that exist between hemicellulose, lignin and glucuronic acids, which impeded chemical modification of hemicellulose by acetylation (Watanabe and Koshijima, 1988; Silveira *et al.*, 2013).

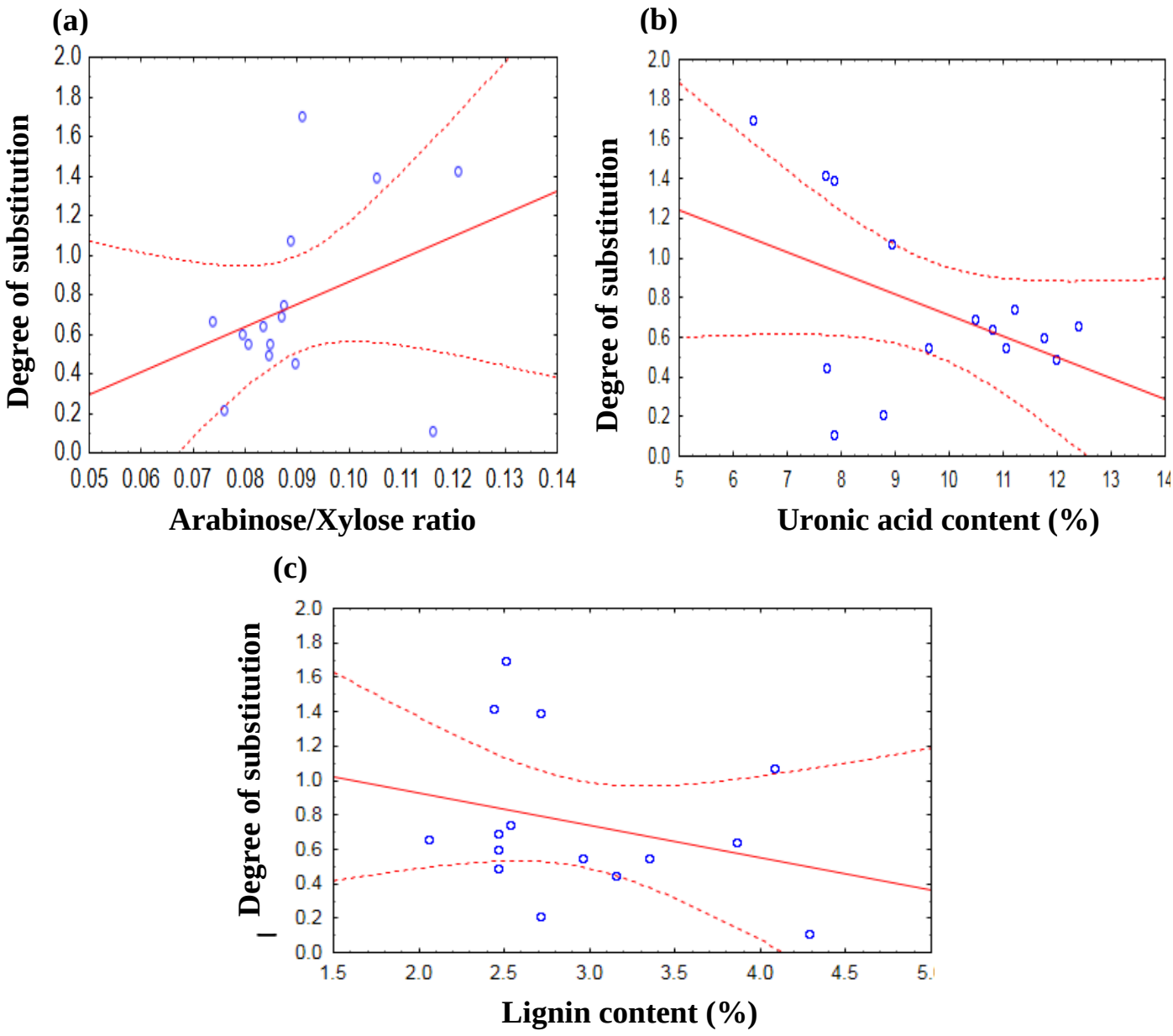


Figure 32: Correlation between the acetylation degree of substitution, hemicellulose uronic acid content and lignin content. . r values for this figure are: (a) +0.35 (b) -0.44 (c) -0.28

The interference of glucuronic acid with hemicellulose modification is evident in other modification processes such as enzymatic modification (Silveira, Stoyanov, Gusarov, Skaf, & Kovalenko, 2013) 2013). Overall, the acetylation process can be enhanced by organosolv treatment conditions. Organosolv treatment can increase the arabinose/xylose ratio and at the same time reduce uronic acids and lignin contents in the hemicellulose extracted, thereby boosting hemicellulose acetylation.

5.5.3. Effect of alkali-organosolv treatment on the hydrophobicity of hemicellulose

The NaOH concentration in the alkali catalysed organosolv treatment, pre-treatment time and interaction of all three factors, had a significant effect ($P < 0.05$) on the hydrophobicity of the AH based films while the EC was not a significant factor (Table 14). It is evident from Figure 33a that among the significant organosolv treatment parameters investigated, the presence of the NaOH in the organosolv treatment had a greater impact on the hydrophobicity than pre-treatment time. The hydrophobicity, measured based on the WCA, varied between 19.88^{o} and 76.26^{o} with changes in organosolv treatment conditions (Table 14 and A1). Therefore, water resistivity of hemicellulose films can be manipulated at the biomass pre-treatment stage by controlling the alkalinity of the pre-treatment medium and the pre-treatment time (Figure 33b, c and d).

Interestingly, the hemicellulose with the highest DS of acetylation did not produce films with the highest hydrophobicity and vice versa (Table 14). The phenomenon confirms that hemicellulose hydrophobicity is not a function of acetylation DS only, contrary to what was previously suggested in literature (Gordobil *et al.*, 2014). The degree of acetylation could only happen to a certain level, leaving other hydroxyl groups unsubstituted. Thus, the unsubstituted hydroxyl groups were available for bonding with water, thereby reducing the hemicellulose film WCA (Gordobil *et al.*, 2014). Similar findings were reported by Minato *et al*., (2007), where the number of hydroxyl groups modified by acetyl groups during acetylation of wood samples did not coincide with the total number of hydroxyl groups available (Minato *et al.*, 2007). However, increasing the acetylation time or the acetylation solvent concentration in order to achieve full acetylation is not an option as this leads to substantial loss of the hemicellulose due to prolonged exposure to acidic solvents (Frisoni *et al.*, 2001).

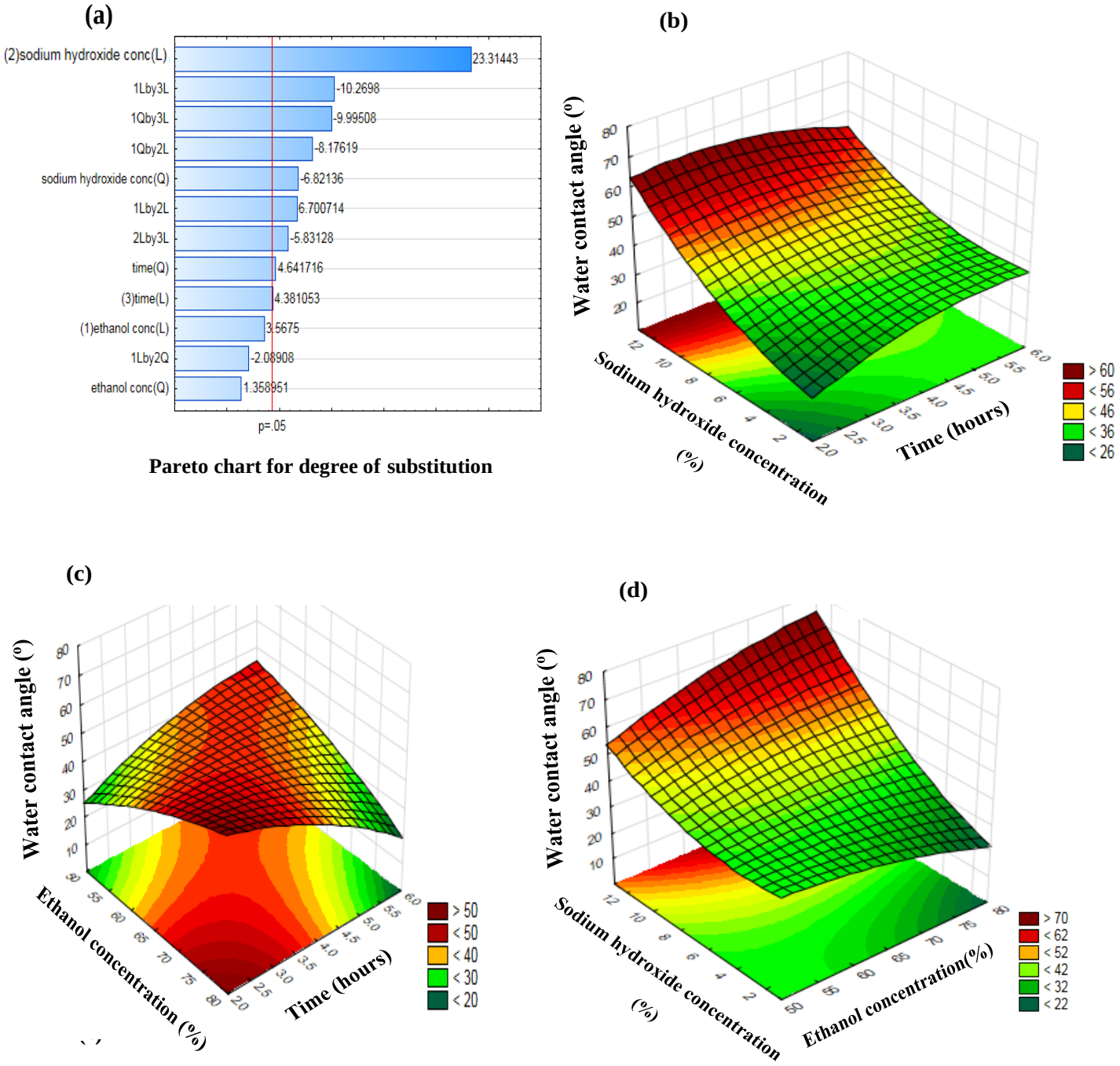


Figure 33: Effect of alkali-organosolv treatment conditions on the hydrophobicity of hemicellulose-based films

It is worth noting that the WCA of the films developed from hemicellulose extracted after organosolv pre-treatment with conditions that inhibited acetylation (Figure 31b and c), that is an increase in NaOH concentration from 1% to 13% (Figure 33b and d) had higher WCA than hemicellulose extracted after pre-treatment with conditions that promoted acetylation. Obviously, such high alkaline conditions may have cleave off arabinose side groups, which are responsible for hydrophilicity in hemicellulose-based films (Li and Pan, 2018). The arabinose side chains tend to

form hydrogen bonds with water, drawing water into the film, thus, reducing the WCA (Akkus *et al.*, 2018; Naidu *et al.*, 2018).

Therefore, the increase in film hydrophobicity can be attributed to the increased debranching of the arabinose side chains (Figure 34a) from the hemicellulose after wheat straw was subjected to extended pre-treatment time and medium alkalinity (Figure 31c). Removal of branches from hemicellulose chains makes them more linear promoting their ability to form a packed structure due to increased intermolecular interaction. The structure inhibits the penetration of water thereby resulting in the hemicellulose being more hydrophobic than hemicellulose with higher branching (Guo *et al.*, 2017). In addition, the presence of polyelectrolyte side chains such as uronic acids in hemicellulose affects the hydrophobicity of hemicellulose. Carboxylic groups on the uronic acid side chains, in particular the COONa ion formed during the sodium hydroxide based extraction, dissociates into ions when hemicellulose is in contact with water making the hemicellulose hydrophilic (Sun *et al.*, 2010; Balto *et al.*, 2016; Flórez-Pardo *et al.*, 2018). Severe pre-treatment conditions (increasing NaOH concentration to up to 13% and reaction time to 6 h) of wheat straw removed the uronic acid chains, thereby increasing hydrophobicity in hemicellulose (Figure 33 b and d).

On the other hand, the effects of lignin on acetylation DS and film WCA were different. The high lignin content in the extracted hemicellulose inversely affected the acetylation (Figure 32c) but increased the WCA of the hemicellulose-based films (Figure 34c). The presence of lignin in hemicellulose meant that some of the hemicellulose hydroxyl groups were covalently bonded to the lignin, minimising the quantity of hydroxyl groups available to attract moisture (Minato *et al.*, 2007). Hence hemicellulose with higher lignin content was less hydrophilic than those with lower lignin content.

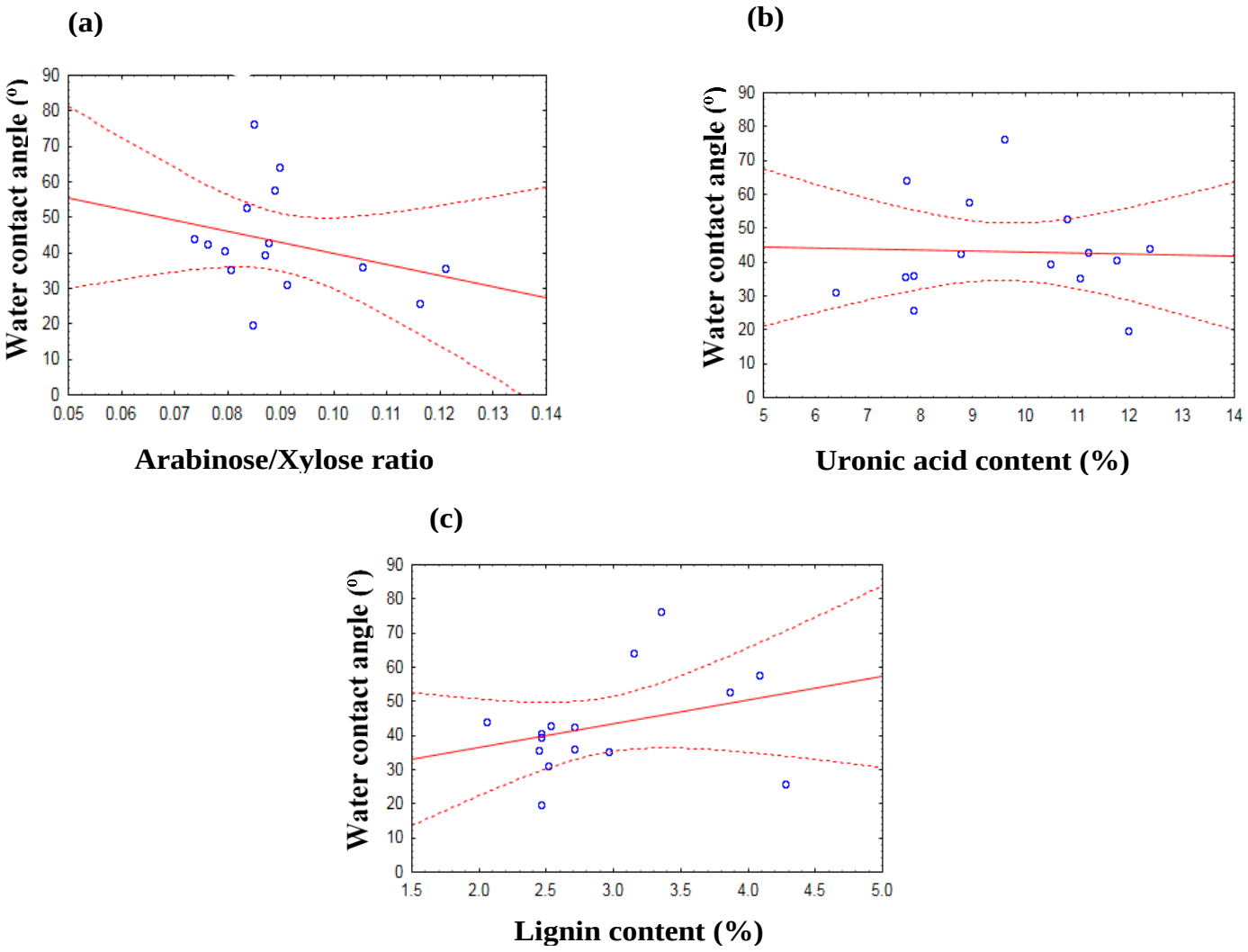


Figure 34: Correlation between water contact angle, hemicellulose arabinose/xylose ratio, uronic acid content and lignin content. r values for this figure are: (a) -0.3 (b) -0.04 (c) +0.32

It can be deduced from these findings that hemicellulose composition with respect to lignin content and arabinose/xylose ratio, as affected by organosolv treatment conditions, had a more significant influence on hemicellulose-based film hydrophobicity than the acetylation process.

This study has shown that hemicellulose composition, which greatly depended on organosolv treatment conditions, was key in influencing the hemicellulose-based film barrier properties to moisture. Manipulation of the organosolv treatment conditions can thus, significantly improve the hydrophobicity of hemicellulose-based films. Therefore, the results support the strategy of manipulating the biomass pre-treatment medium to engineer performance of hemicellulose-based films. However, manipulating the alkaline organosolv treatment conditions to achieve a high degree of hydrophobicity in the hemicellulose-based films may negatively affect hemicellulose yield from the wheat straw in the subsequent alkaline extraction process.

5.5.4. Optimisation of the alkali-organosolv treatment process

The relationship between the organosolv treatment response variables and the significant dependent variables is shown by model Equations 19-23.

$$Hemicellulose\ yield\ (\%) = 302.413 - 7.8293X_1 + 0.0614X_1^2 + 0.9624X_3^2 - 0.3238X_2X_3 \quad (19)$$

$$Arabinose\ to\ xylose\ ratio = 0.000104X_2 + 0.000404X_2^2 \quad (20)$$

$$Uronic\ acid\ content\ (\%) = 5.43921X_2 - 0.19429X_2^2 \quad (21)$$

$$Water\ contact\ angle\ (^\circ) = -777.66 + 23.572X_1 - 0.173X_1^2 + 33.516X_2 + 148.932X_3 - 1.091\ X_3^2 - 1.007X_1X_2 + 0.008X_1^2X_2 - 3.996X_1X_3 + 0.028X_1^2X_3 - 0.439X_2X_3 \quad (22)$$

$$DS = -19.2551 + 0.7790X_1 - 0.0072X_1^2 + 0.099X_2^2 - 0.072X_1X_2 - 0.0015\ X_1X_2^2 + 0.0008X_1^2X_2 \quad (23)$$

X_1, X_2, X_3 and DS are EC, NaOH concentration, time and degree of substitution respectively.

Due to the contradicting effects of organosolv treatment conditions on acetylation and film hydrophobicity (Section 5.5.2 and 5.5.3), two optimum organosolv treatment conditions were obtained (OPT1 and OPT2). OPT1 organosolv treatment conditions enhanced the acetylation process and the second optimisation procedure (OPT2) focused on minimising the DS required with the aim of improving film hydrophobicity. The optimum organosolv treatment conditions, OPT1, were 50% EC, 1% NaOH concentration and pre-treatment time of 6 h whereas optimum conditions OPT2 were 80% EC, 13% NaOH concentration and pre-treatment time of 5 h. Results for the experimental validation of the optimum conditions and the model predictions are shown in Table 15. Hemicellulose compositions at both optimum conditions were within the 95% confidence level of the model predicted values.

Table 15: Experimental results and model predictions of the hemicellulose properties for undelignified hemicellulose and hemicellulose from optimum conditions

Parameter	Hemicellulose yield (%)	Uronic acid content (%)	Lignin content (%)	Arabinose/xylose ratio	Water contact angle (°)	Acetylation degree of substitution
Undelignified hemicellulose	78.11	12.28	6.42	0.139	34.21	1.2
OPT1						
Experimental results	52.96	7.38	3.37	0.093	39.90	1.7
Model prediction	56.34	6.84	3.44	0.095	48.72	1.81
OPT2						
Experimental results	7.89	9.26	4.39	0.088	68.1	0.4
Model prediction	9.95	8.88	3.79	0.087	62.98	0.29

OPT: optimum

Both optimum alkali-organosolv treatment conditions successfully minimised the arabinose/xylose ratio, UAC and lignin content in the hemicellulose as highlighted by their quantities in OPT1 and OPT2 hemicellulose when compared to hemicellulose from unpre-treated biomass (Table 15). The alkali-organosolv treatment conditions tailor-made the WCA of the hemicellulose-based films. Hemicellulose films made from hemicellulose extracted from unpre-treated biomass had the least WCA (Table 15 and A2). As noted in Table 15, the hydrophobicity increased with an increase in the severity of alkali-organosolv treatment that is unpretreated < OPT1< OPT2. Similarly, surface roughness (Figure. A1) was observed to follow the same trend indicating that roughness of the films increased with pre-treatment severity. OPT2 conditions can be considered as severe alkali-organosolv treatment conditions, with respect to the NaOH concentration in the pre-treatment solvent, when compared to OPT1 conditions.

The OPT1 conditions yielded hemicellulose with more arabinose side chains than those obtained at OPT2 conditions (Table 15). Therefore, the hemicellulose from OPT1 had more hydroxyl groups available for acetylation. However, hemicellulose recovered at OPT2 conditions, which had a lower degree of acetylation when compared to hemicellulose obtained at OPT1 conditions, generated films with higher WCA compared to films generated from both hemicellulose obtained at OPT1 and the hemicellulose from the untreated biomass. Thus, there is clear evidence that acetylation was not effective in replacing all hydroxyl groups in the hemicellulose. The conditions at OPT2 effectively cleaved more arabinose side chains than OPT1, leaving a few hydroxyl groups that were substituted by acetyl groups during acetylation hence the lower DS in OPT2 hemicellulose than in OPT1 hemicellulose (Table 15). Apart from the removal of arabinose, the presence of lignin exhibit a similar phenomenon (Minato *et al.*, 2007; Egüés *et al.*, 2014). Therefore, a combination of higher lignin content and lower hydroxyl groups in hemicellulose obtained at OPT2 could have inhibited acetylation but on the other hand, promoted hydrophobicity of the films.

5.5.5. Effects of alkali-organosolv treatment conditions on mechanical properties of hemicellulose-based films

When hemicellulose-based films were made without any reinforcements, OPT2 hemicellulose-based films had the highest tensile strength, Young's modulus, and strain at break when compared to OPT1 hemicellulose films (Figure 35). The higher lignin content in OPT2 hemicellulose, when compared to OPT1 hemicellulose, could have contributed to better mechanical properties in OPT2 than in OPT1. Lignin has been reported to improve the structural strength of both bio-composites and synthetic polymers (Peng *et al.*, 2018). However, on addition of the ACNC as a reinforcement, the tensile strength and Young's modulus of OPT1 films improved significantly and exceeded that of OPT2 with and without reinforcement (Figure 35a and b). A higher acetyl content in OPT1

hemicellulose (DS 1.7) than in OPT2 (DS 0.4) could have positively affected the mechanical properties of OPT1 due to enhanced compatibility between the matrix and reinforcing material (ACNC DS 2.34). However, due to a lower WCA in OPT1 hemicellulose-based films when compared to OPT2 hemicellulose-based film, these results contradict the findings by Wang, Kabir & Lau (2014). Their work highlighted that the higher the hydrophobicity of a matrix, the more compatible it is with a hydrophobic filler (Wang *et al.*, 2014).

As the reinforcing ACNC content in AH films increased, it negatively affected the percent elongation of the films (Figure 35a and d), with a higher impact on OPT2 hemicellulose-based films than OPT1 hemicellulose-based films. However, the effect of the addition of nanocellulose to both OPT2 hemicellulose and OPT1 hemicellulose-based films was not statistically significant as highlighted by the letters in Figure 35. Nanocellulose improves the stiffness of material but compromises their elasticity, hence, the decrease in hemicellulose-based film elongation with an increase in ACNC loading (Bagheriasl *et al.*, 2015). OPT1 hemicellulose-based films were more elastic than OPT2 hemicellulose-based films (Figure 35d), this could be because of higher arabinose content and DS in OPT1 films (Fang *et al.*, 2000; Egüés *et al.*, 2014). Therefore, the alkali-organosolv treatment process has an impact on the interaction between hemicellulose and nanocellulose in a composite film.

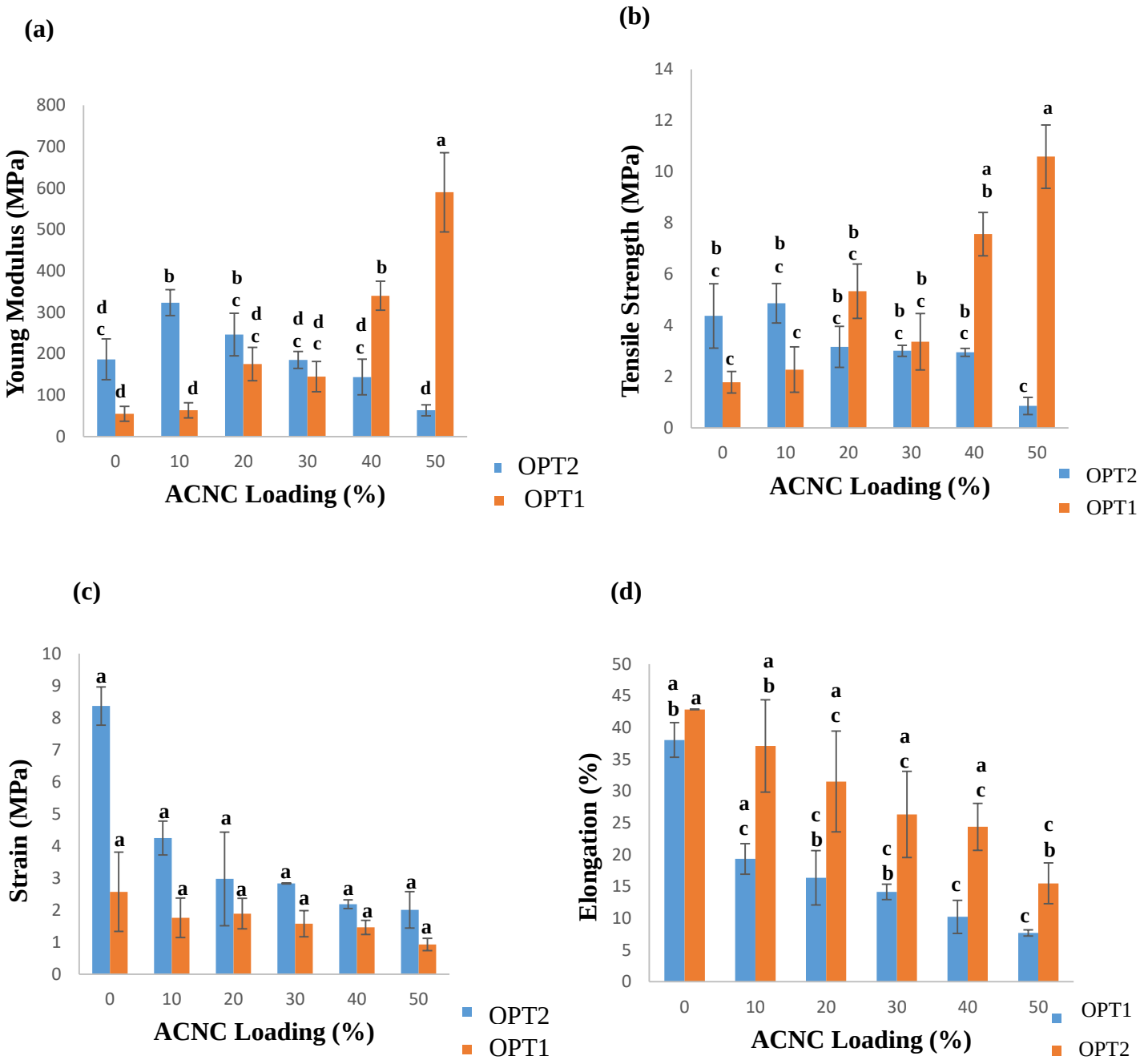


Figure 35: Effect of acetylated nanocellulose (ACNC) loading on the mechanical properties films produced from hemicellulose extracted from wheat straw that were subjected to mild optimum (OPT1, 1% NaOH/50% ethanol/6 h) and severe (OPT2, 13% NaOH/80% EC/5 h) conditions. Letters denote the mean significant differences.

5.6. Conclusions

Alkali-based organosolv treatment conditions significantly affected hemicellulose acetylation, hemicellulose filler interaction and film hydrophobicity. Optimisation of the alkali-based organosolv treatment conditions established two optimal conditions. The organosolv treatment optimal conditions, OPT1 (1% NaOH, 50% EC, 6 h), promoted acetylation and the reinforcing efficiency of ACNC in hemicellulose films but negatively affected the film hydrophobicity, whereas, OPT2 (13% NaOH, 80% EC, 5 h) minimised acetylation DS and hemicellulose-filler interaction but improved hydrophobicity in hemicellulose-based films. Therefore, biomass pre-treatment conditions play an important role in influencing the response of hemicellulose to modification by acetylation and in turn the mechanical properties and hydrophobicity of hemicellulose-based films. Thus, there is potential in combining the alkali-catalysed organosolv pre-treatment of biomass and acetylation processes to produce biopolymers with diverse mechanical and water sensitivity that can be used in food packaging development. OPT1 hemicellulose can be used to develop films and OPT2 hemicellulose can be used for formulation of coatings for fatty food packaging.

6. DEVELOPMENT OF HEMICELLULOSE-BASED FILMS FOR ACTIVE FOOD PACKAGING APPLICATION

Titles of papers contributing to the chapter

1. Biocomposites containing hemicellulose, pectin and nanocellulose: Physicochemical properties and potential application as a packaging material with mango peel antioxidant release into fatty foods
2. Reinforcement efficiency of acetylated nanocellulose as a filler in acetylated hemicellulose films.
3. Enhancing functional properties of acetylated hemicellulose films for food packaging using ACNC and PCL coatings

Dissertation objectives fulfilled in this chapter

Chapter 6 successfully met the requirements for objectives 3, 4, 5 and 6. Reinforcing acetylated hemicellulose with ACNC and coating the AH/ACNC films with PCL can produce films with similar hydrophobicity and mechanical properties to LDPE films. In addition, the hemicellulose-based films can be utilised as a substitute active packaging material to LDPE films for short term storage of food. In this chapter biocomposites were developed by blending hemicellulose pectin and nanocellulose and the resulting biocomposite mechanical properties and migration into food, were evaluated. The same was also done for AH and ACNC. Briefly, the effects of ACNC as a reinforcing material in AH, were investigated with the aim of improving applicability of the films in food packaging (as a substitute for LDPE films). The effectiveness of ACNC as a reinforcement material for AH was evaluated both experimentally and by the use of theoretical models. Mango polyphenols were incorporated into the AH/ACNC films and hemicellulose/pectin/nanocellulose films and the films potential release of antioxidants into food simulants was evaluated both experimentally and by modelling with Migratest EXP software.

Summary of findings

Hemicellulose-based films developed had mechanical properties and hydrophobicity similar to that of LDPE films. Both the AH/ACNC films and the hemicellulose/pectin/cnc films were least soluble in the fatty foods when compared to the other simulants. The films were capable of releasing the mango peel antioxidants into the food simulants, with the highest release being into the fatty foods. In addition, the rate of release of antioxidants by the hemicellulose-based films in

response to storage conditions was higher than that for LDPE films. Therefore, hemicellulose-based food packaging material is a better alternative to LDPE active packaging for short term storage of food.

Authors: Lindleen Mugwagwa, Annie Chimphango

Summary of authors' contributions

Lindleen Mugwagwa was responsible for planning, performing experiments and modelling. Both authors did analysis and interpretation of the results. Lindleen contributed 80% of the work while Annie Chimphango did 20% of the work in this chapter.

6.1. Paper Title: "Biocomposites containing hemicellulose, pectin and nanocellulose: Physicochemical properties and potential application as a packaging material with mango peel antioxidant release into fatty foods"

Submitted to the journal of Food packaging and shelf life (impact factor 3.67)

6.2. Abstract

Hemicellulose/pectin/nanocellulose biocomposite films were evaluated as active packaging material for fatty foods. The effects of varying hemicellulose/pectin content (0% - 100%) and incorporating 25% nanocellulose on the films' Young's modulus, tensile strength, percent elongation and solubility in the fatty simulant were studied. Polyphenols were encapsulated in the biocomposite and the biocomposite's antioxidant release into the fatty simulant was assessed at 5 - 40 ^{o}C for 48 h. Biocomposites with a pectin content >50% (pectin-based films) had improved tensile strength (68.93 vs 2.73 MPa), Young's modulus (6064.94 vs 139.08 MPa) and were less soluble in the simulant (4.85 vs 39.96%) than those with hemicellulose content >50% (hemicellulose-based films). However, hemicellulose-based biocomposites had higher percent elongation (12.99% vs 4.73%) than pectin-based films. The biocomposite released up to 0.021 mg/h of the encapsulated polyphenols resulting in an increase in the simulant's antioxidant activity by up to 22.5%. Therefore, the films can be used as active packaging for fatty foods.

Keywords: Active packaging; Hemicellulose; Pectin, Nanocellulose; Mango polyphenols;

Migration modelling

6.3. Introduction

The development of biopolymer based food packaging material that can replace petroleum based packaging is gaining momentum in the food packaging industry (Mikkonen and Tenkanen, 2012; Kanatt and Chawla, 2018). Biobased films can be engineered to achieve certain mechanical (tensile strength, Young's modulus) and functional properties such as minimal solubility in food media (Nešić *et al*., 2020; Ramos *et al*., 2018). Potential strategies for enhancing the functionality of the packaging materials include incorporating polymers with complementary functional properties into the material.

Biopolymers such as hemicellulose, cellulose, and pectin are attractive raw materials for food packaging films due to their abundance, low toxicity, and biodegradability (Agustin, Nakatsubo, & Yano, 2018; Espitia, Du, Avena-Bustillos, Soares, & McHugh, 2014; Mendes *et al*., 2017). Arabino-4-O-glucurunoxylans hemicellulose, are heteropolymers consisting of a xylose backbone and side chains of uronic acids and arabinose (Peng, Peng, Xu, & Sun, 2012). Cellulose is a biopolymer chain constituting of glucose units linked by β-1,4 glycosidic bonds (Lee, Hamid, & Zain, 2014). Pectin is polysaccharide chain composed mainly of GA units and a few branching of neutral and acidic sugars (Klinchongkon *et al.*, 2016). Utilisation of the films developed from the individual aforementioned biopolymers for food packaging is hindered by the films' low tensile strength and Young's modulus, brittleness, and high solubility in food media when compared to conventionally used packaging films such as low density polyethylene films (Chen *et al*., 2015; Mendes *et al*., 2019; Sartori, Feltre, do Amaral Sobral, Lopes da Cunha, & Menegalli, 2018). The high dissolution rate of hemicellulose and pectin films in food containing water is attributed to the abundance of hydroxyl groups in the structure of the two polymers (emanating from side chains) which form hydrogen bonds with water. Consequently, accumulation of moisture in the biopolymer also contributes to poor mechanical properties (Koroskenyi and McCarthy, 2001; Sucheta *et al.*, 2019). On a positive note, side chains such as arabinose in the hemicellulose and pectin structure contribute to film flexibility. Nanocellulose forms brittle films due to the absence of branching in the nanocellulose structure and because of the overall stiffness of nanocellulose (Lee *et al*., 2014). Therefore, in order to take advantage of the unique properties of each of the biopolymers, the biopolymers can be blended to develop a biocomposite film with enhanced mechanical (Young's modulus, tensile strength and percent elongation) and functional properties (for example moisture barrier properties) (Huang *et al*., 2018; Mendes *et al*., 2017; Norcino, de Oliveira, Moreira, Marconcini, & Mattoso, 2018).

Previous studies have primarily focused on reinforcing either hemicellulose- or pectin- based films with nanocellulose, with the aim of improving the films' mechanical and physical properties

such as Young's modulus, tensile strength and water vapour barrier properties (Chaichi, Hashemi, Badii, & Mohammadi, 2017; Egüés, Stepan, Eceiza, & Toriz, 2014; Lee *et al*., 2014a). For instance, the tensile strength of hemicellulose-based films was reported to increase from 8.05 MPa to 11.15 MPa when nanocellulose content of the films increased from 0% to 6% (Huang *et al.*, 2018). On the other hand tensile strength and Young's modulus of pectin films increased from 7.12 MPa to 13.15 MPa when 5% nanocellulose was added (Chaichi *et al.*, 2017). Blending the three biopolymers has the potential to further improve the physicochemical properties of the biocomposites beyond that of biocomposites with two biopolymers.

Notably, assessing the stability of the films when in contact with specific food media is crucial, if the film is to be used as a food packaging material, and should be evaluated alongside the mechanical and barrier properties. Biocomposites formed by blending the three biopolymers are expected to remain stable in fatty foods as the individual polymers are insoluble in 95% ethanol (Koubala *et al*., 2008; Peng *et al*., 2012). Coincidentally, 95% ethanol is used as a fatty food simulant (Chen, Lee, Zhu, & Yam, 2012; López De Dicastillo *et al*., 2011), substituting the use oils and fats in testing food packaging stability. This is because oils and fats adhere to the packaging material, making it difficult to test the properties of the film (Feigenbaum *et al.*, 2000). The phenomenon (limited solubility in ethanol), implies that films made from these polymers either on their own or in blends (biocomposites) can remain stable when in contact with fatty foods, hence they are suitable for packaging fatty foods.

More importantly, research in development of food packaging material has passed the phase of formulating packaging material with the aim of enhancing the material's barrier and mechanical properties only. Instead, food packaging materials are being integrated with active compounds (active food packaging) to produce a packaging material that can maintain food quality during storage (Muriel-Galet *et al.*, 2015). With conventional packaging, the quality of food deteriorates over time due to the build-up of oxidising agents, free radicals and microorganisms in the packaged food (Manohar *et al.*, 2015). Temperature and time are the major factors that facilitate the accumulation of the aforementioned agents causing food spoilage (Manohar *et al.*, 2015). Antioxidants can be added to the food to prevent or slow down oxidation processes and growth of microorganisms that cause food spoilage. However, the major drawback of adding antioxidants directly to food is the possibility of instant deactivation of the antioxidants by the food components. Moreover, increasing the concentration of the active compounds in the food to compensate for the deactivated antioxidants can result in the antioxidants exceeding allowable limits. Consequently, the antioxidants can become a health hazard to the consumers (Ortiz-Vazquez *et al.*, 2011). Therefore, integrating the active compounds into the food packaging structure can be a favourable strategy that

would ensure a slow and just in time release of the antioxidants into the food (Ortiz-Vazquez *et al.*, 2011; Dias *et al.*, 2018), ultimately ensuring that the antioxidants remain functional and are only released into the packaged food in response to variations in storage conditions such as temperature and time. Hence, an active packaging material is a better option than the direct addition of antioxidants to food when aiming to maintain the quality of food over a long time (Uzunlu and Niranjan, 2017).

In this study, films with different ratios of hemicellulose to pectin content were formulated, and the mechanical properties and solubility of biocomposites in fatty foods were compared with those of films made from the individual polymers. Furthermore, the study assessed the effect of adding nanocellulose as a reinforcement to both hemicellulose-based films and pectin-based films on the mechanical properties and solubility of the films in fatty simulant. Additionally, mango peel polyphenols were integrated into the biocomposite films and their release into the food simulant over time and under varying temperatures was evaluated. The migration behaviour of the polyphenols from the biocomposite films into the fatty simulant was mathematically modelled and compared to the migration predictions made by the Migratest EXP software (FABES Forschungs-GmbH) (Zülch and Piringer, 2010).

6.4. Materials and methods

6.4.1. Chemicals

NaOH, ethanol, HCl, and sorbitol were sourced from Kimix Chemical & Lab Supplies (Cape Town, South Africa). Ammonium oxalate, oxalic acid, glucuronic acid, GA, Folin Ciocalteu reagent, DPPH sodium carbonate, sugars standards (arabinose and xylose), carbazole, gallic acid, and acetic acid, were purchased from Sigma Aldrich (Germany). University of Maine supplied the nanocellulose nanocrystals (nanocellulose crystals, freeze dried, 100 - 150 nm, 1.5 g/cm^3). All the chemicals were of analytical grade.

6.4.2. Hemicellulose, pectin, and polyphenols extraction methods

Hemicellulose was extracted from wheat straw using OPT1 conditions and its composition analysed as described in section 5.4.3. Pectin and polyphenols were recovered from mango peels using the three-stage process optimum conditions highlighted in section 4.10.1. The composition and properties of the recovered products were analysed using the method highlighted in section 4.4

6.4.3. Development of biocomposite films composed of hemicellulose, pectin and nanocellulose

Composition of hemicellulose and pectin used in developing the films is given in table B1 and B2. The hemicellulose- and pectin-based films, with hemicellulose/pectin ratios highlighted in

Table 16, were developed by solvent casting. Sorbitol, approximately 25% of the total film solid weight (500 mg), was added as plasticizer. The hemicellulose, nanocellulose, pectin and sorbitol were dissolved in 40 mL of water by heating the mixture for 30 min at 50 ^{o}C in a shaking water bath. The homogenous mixture was poured into 80 mm plastic Petri dishes and dried for three days at 25 ^{o}C. An Elcometer (model 456CFSS) was used to measure film thickness.

Table 16: Hemicellulose to pectin ratio used for developing hemicellulose-based films and pectin-based films

Film	Hemicellulose content (mg/500 mg)	Pectin content (mg/ 500 mg)	Hemicellulose/pectin ratio
Film 1	500	0	5:0
Film 2	400	100	4:1
Film 3	300	200	3:2
Film 4	250	250	1:1
Film 5	200	300	2:3
Film 6	100	400	1:4
Film 7	0	500	0:5

Hemicellulose/pectin/nanocellulose films were made using the same method described in section 6.4.3 with the only difference being the addition of 25% nanocellulose (based on the total film solids) to the films. The quantity of nanocellulose used as reinforcement was adopted from Peng, Ren, Zhong, & Sun, (2011).

6.4.4. Determination of physicochemical properties of biocomposite films

6.4.4.1. Mechanical properties of hemicellulose-based films and pectin-based films

The films were tested for tensile strength, Young's modulus and percent elongation using a LRX Instron tensile tester supplied by LLOYD instruments. The equipment was maintained at a head speed of 50 mm/min and gauge length of 27.3 cm during the analysis of mechanical properties. Specimens of the films used for the analysis were cut to a length of 6 cm and a width of 1 cm.

6.4.4.2. Determination of film solubility in the fatty food simulant

The solubility of hemicellulose- and pectin-based films in the fatty food simulant was determined by a method reported by Traistaru, Rivis, Moldovan, & Menelaou (2013). The rate of dissolution of the films in the fatty food simulant was tested using 95% ethanol. The specimens used for solubility testing were prepared by cutting the films into square strips measuring 2 cm × 2 cm. The initial weight of the strips was measured before inserting the strips in 30 mL of 95%

ethanol. The experiment was carried out in plastic falcon tubes incubated in a shaking water bath set at 25 ^{o}C for 24 h. The films were then oven dried for a day at 40 ^{o}C prior to measuring the final weight of the film. The solubility of the film in the simulant was calculated as the percentage of the film that dissolved in the simulant.

6.4.5. Assessing the active properties of hemicellulose/pectin/nanocellulose films doped with mango polyphenols

6.4.5.1. Determination of the antioxidant release potential of hemicellulose/pectin/nanocellulose films

Hemicellulose/pectin/nanocellulose films with a hemicellulose/pectin ratio of 300:200 and 25% nanocellulose were developed according to the method outlined in section 6.4.3. About 100 µL of the mango polyphenol extract in ethanol was added to the hemicellulose/pectin/nanocellulose mixture before solvent casting was performed using the method specified in section 6.4.3. The mango polyphenols were extracted from mango peels using the method provided in section 4.10.1. and analysed using the methods provided in section 4.4. The antioxidant release studies were conducted according to the method by Yu *et al*. (2017). Films with dimensions, 2 cm x 2 cm, were immersed in 30 mL of the fatty simulant (95% ethanol). The experiment was carried out in 50 mL plastic falcon tubes. The antioxidant release of the film into the food simulant was tested at 5 ^{o}C, 25 ^{o}C and 40 ^{o}C under stirring. A sample of the simulant was taken and analysed for antioxidant activity and polyphenol content at 4-hour intervals according to the methods presented in section 4.4.3.

6.4.5.2. Modelling the antioxidant release by hemicellulose/pectin/nanocellulose films

The release of mango polyphenols into the fatty food simulant by the hemicellulose/pectin/nanocellulose films was modelled using Migratest EXP software (Zülch and Piringer, 2010). Migratest EXP estimates the release of migrants from packaging material based on Equation 45 (Gavriil *et al.*, 2018).

$$\frac{\partial C_P^H}{\partial t} = D_P^H \frac{\partial^2 C_P^H}{\partial x^2} \qquad (45)$$

where:

$\frac{\partial C_P^H}{\partial t}$ is the rate of migration of the polyphenols from the hemicellulose/pectin/nanocellulose film.

D_P^H is the diffusion coefficient of the polyphenols.

$\frac{\partial^2 C_P^H}{\partial x^2}$ is the rate of change of polyphenol concentration in the film as a function of the distance the polyphenols travel from the film into the simulant (x).

The diffusion coefficients of the mango peel polyphenols were calculated using Equation 46 (Yu *et al.*, 2017).

$$D = \left(\frac{kL_P}{4}\right)^2 \pi \qquad (46)$$

where:

D is the diffusion coefficient.

L_P is a length calculated by dividing the film thickness by two

k is the slope of the graph; time vs $\frac{M_{F,t}}{M_{F,\infty}}$

$M_{F,t}$ is the concentration of mango polyphenols that migrated into the simulant at a specific time $M_{F,\infty}$ is the maximum concentration of mango peel polyphenols that migrated into the simulants.

The partition coefficients of the hemicellulose/pectin/nanocellulose films were calculated according to Equation 47.

$$K_P = \left(\frac{C_P}{C_S}\right) \qquad (47)$$

where:

K_P is the partition coefficient

C_P is the concentration of the mango polyphenols remaining in the hemicellulose/pectin/nanocellulose film after 2 days

C_s is the concentration of mango polyphenols that migrated into the simulant after the 2 days (Gavriil *et al.*, 2018).

In order to model the antioxidant release, the following specifications were used: fatty food simulant volume- 30 mL, temperatures of 5 ^{o}C, 25 ^{o}C and 40 ^{o}C, and time ranging from 0-48 h. The hemicellulose/pectin/nanocellulose/polyphenol films length, width and thickness were set as 2 cm x 2 cm x 0.0064 cm. The density of hemicellulose/pectin/nanocellulose film was specified as 1.435 g/cm^3. This density value was experimentally calculated from the densities of hemicellulose, pectin and nanocellulose. The mango polyphenols molecular weight was specified as 300.07 g/mol, which is the average of the standard molecular weights of the different polyphenols in the mango peel polyphenol extract (Shivapuji, 2019).

6.4.6. Structural characterisation of hemicellulose-based films and pectin-based films

6.4.6.1. Determination of interaction among components in hemicellulose-based films and pectin-based films

Images of biocomposite films made of hemicellulose, pectin and nanocellulose were captured at magnifications of 150 and 1.25 using a Carl Zeiss Merlin Field Emission Scanning Electron Microscopy (FESEM) equipped with a GEMINI II column. Film preparation prior to taking the images involved coating the films (1.5 cm x 1.5 cm) with gold and sticking the film on a stub using a double-sided tape. The in-lens detector used for imaging was set at the following conditions: working distance = 6.7 mm, probe = 100 Pa and EHT = 3.00 kV.

6.4.6.2. Determination of functional groups in hemicellulose-based films and pectin-based films

A Nexus model Fourier Transform Infrared (FTIR) spectrometer fitted with a Golden Gate diamond single reflectance ATR sampling accessory (Thermo Scientific, Nicolet, USA) was used to determine the chemical composition of the films. The spectrometer was set to measure the absorbance of the biocomposite films from a wavelength of 650 to 4000 cm^{-1} at a resolution of 8 cm^{-1} by carrying out 64 co-added scans. The FTIR spectra were developed using Origin 6 software (OriginLab corporation, USA).

6.4.7. Statistical analysis

The effects of temperature and time on the release of antioxidants by the hemicellulose/pectin/nanocellulose films and the antioxidant activity of the fatty simulant were determined by Analysis of Variance (ANOVA). The differences in mean values of the experimental data were analysed using the Duncan multiple stage procedure. The Duncan multiple state process is a post hoc test which determines if there is a significant difference between any two means (Tallarida and Murray, 1987). All the above-mentioned analyses were done using Statistica version 7, 13.2 software.

6.5. Results and discussions

Pectin can be added to hemicellulose-based films as a filler to produce thin biocomposite films, while hemicellulose fillers can be added to pectin-based films to create thick biocomposite films. The results show that biocomposites films with different ratios of hemicellulose to pectin content had thicknesses ranging from 40.05 µm to 66.12 µm (Table 17). The thicknesses of the films were within the ideal range (38 - 76 µm) for films used in food packaging applications (Pavlath, 2009). The results in Table 17 show that hemicellulose-based films were relatively thicker (at 62.74 µm to 66.12 µm for Film 1 to 3) than pectin-based films (at 40.05 µm to 58.32 µm for Film 5 to 7). Furthermore, the addition of hemicellulose as a filler in pectin films (hemicellulose content from

0% to 50%, hemicellulose/pectin ratio from 0:5 to 1:1) increased the films' thickness by 50%, while a pectin filler (pectin content from 0% to 50%, hemicellulose/pectin ratio from 5:0 to 1:1) in hemicellulose films reduced the hemicellulose-based film thickness by 9.09% (Table 17) The increase in thickness of pectin films upon adding hemicellulose is due to the replacement of the tightly packed pectin particles with the loosely bound hemicellulose particles (Table 18). Consequently, the reduction in hemicellulose films' thickness upon adding pectin filler is due to the reduction in voids in the film by introducing the pectin particles (Table 18).

The ability to manipulate hemicellulose- and pectin-based film thickness can diversify the application of hemicellulose/pectin biocomposites in food packaging. For instance, thin films, as is the case with hemicellulose-based films enriched with pectin as a filler, (when compared to pure hemicellulose films) are particularly important for application as edible films or coatings because they would quickly disintegrate when consumed. The rapid breakdown of the edible films and coatings is a crucial property that minimises the film from interfering with the flavours of the coated food (Pavlath, 2009). On the other hand, pectin-based films enriched with hemicellulose would be more attractive as active packaging for fatty food applications than thin films (pure pectin films). The thicker films have the ability to encapsulate and release bioactive agents into the packaged food at a slower rate than thinner films. The slow release of the encapsulated bioactive substances by such films is attributed to the longer time the active compounds have to diffuse through the thick layer to reach the surface of the food (Rossi-Márquez *et al.*, 2009).

Table 17: Hemicellulose-based and pectin-based films' thickness as affected by pectin and hemicellulose filler content respectively

Film*	**Film thickness (µm)**
Film 1	$66.12^{a} \pm 5.13$
Film 2	$57.26^{b} \pm 4.00$
Film 3	$62.74^{a} \pm 6.69$
Film 4	$60.11^{ab} \pm 2.50$
Film 5	$58.32^{c} \pm 4.93$
Film 6	$52.58^{d} \pm 6.91$
Film 7	$40.05^{d} \pm 5.52$

*Biocomposite containing different hemicellulose to pectin ratios, thus Film 1: (5:0), Film 2: (4:1), Film: 3 (3:2), Film 4: (1:1), Film 5: (2:3), Film 6: (1:4), Film 7: (0:5). Five measurements were carried out on the film. The values are means of three measurements. Values with different letters are significantly different ($p < 0.05$).

6.5.1. Effect of hemicellulose/pectin ratio and nanocellulose reinforcement on film solubility in the fatty food simulant

The presence of the hemicellulose was the major contributing factor to the solubility of the biocomposite in the fatty food simulant. The hemicellulose-based films (Films 1-3) were more soluble in fatty food simulants than pectin-based films (Films 5-7), as depicted in Figure 36. Pure hemicellulose films (Film 1) were the most soluble films, exhibiting 39.97% solubility and the least soluble were the pure pectin films (Film 7) showing 4.85% solubility Figure 36. Evidently, the solubility of the composite films in the simulant was influenced by the ratio of the polymers in the biocomposite films as highlighted by the pareto charts in Figure B1. Notably, increasing pectin content from 0% to 67% in the hemicellulose-based films significantly decreased ($p < 0.05$) the solubility of the composite film in the fatty food simulant from 39.96% to 31.12% (Figure 36). In contrast, increasing the hemicellulose loading in pectin-based films from 0% to 67% (Film 7 to 4), led to an increase in the films' solubility in the fatty simulant from 4.85% to 21.39% (Figure 36). In addition, when 67% of the film components was hemicellulose, the solubility difference between the pure pectin film and the pectin-based composite was 16.54% whereas when 67% of the components is pectin, the solubility difference between the pure hemicellulose film and the hemicellulose-based composite was 8.84%. Thus, blending hemicellulose and pectin to form a biocomposite can be a strategy to manipulate the functional properties of the biocomposite films, particularly, solubility in fatty foods.

The differences in solubility between pure hemicellulose and pure pectin films can be attributed to the molecular weights of the two polymers. The lower solubility of the pure pectin films in the fatty simulant when compared to the pure hemicellulose films is due to pectin (352 879 g/mol) having a higher molecular weight than hemicellulose (39 221 g/mol) (Table B1 and B2). Polysaccharide chains with higher molecular weight tend to exhibit a higher particle to particle interaction when compared to low molecular weight polysaccharides. Increased particle interaction in films made by polysaccharide chains of higher molecular weight minimise solvent penetration into the film when the film is in contact with the fatty simulant, thereby limiting film solubilisation. Moreover, there is a direct correlation between particle size and molecular weight of polymers. Hence, the pectin (polymer chain has larger particles) diffuse at a slower rate into the solvent than hemicellulose (polymer chain has smaller particles), leading to a lower dissolution rate of pectin-based films when compared the hemicellulose-based films (Guo *et al.*, 2017). In addition, the higher surface charge of pectin lowers the pectin interaction with polar solvents like ethanol, making it more insoluble in ethanol when compared to hemicellulose (Balto *et al.*, 2016). The higher UAC (Table B2) of pectin (85.5%) is the primary contributor to the higher surface charge in the pectin

when compared to hemicellulose (7.14%). Notably, the hemicellulose that was used in formulating the films was mainly composed of neutral sugars (Table B1). Partly, the dissolution of the films in the fatty simulant can be attributed to the hydrogen bonding of the films' components (Figure B1) and water in the simulant (5% water) (Fortunati *et al.*, 2012).

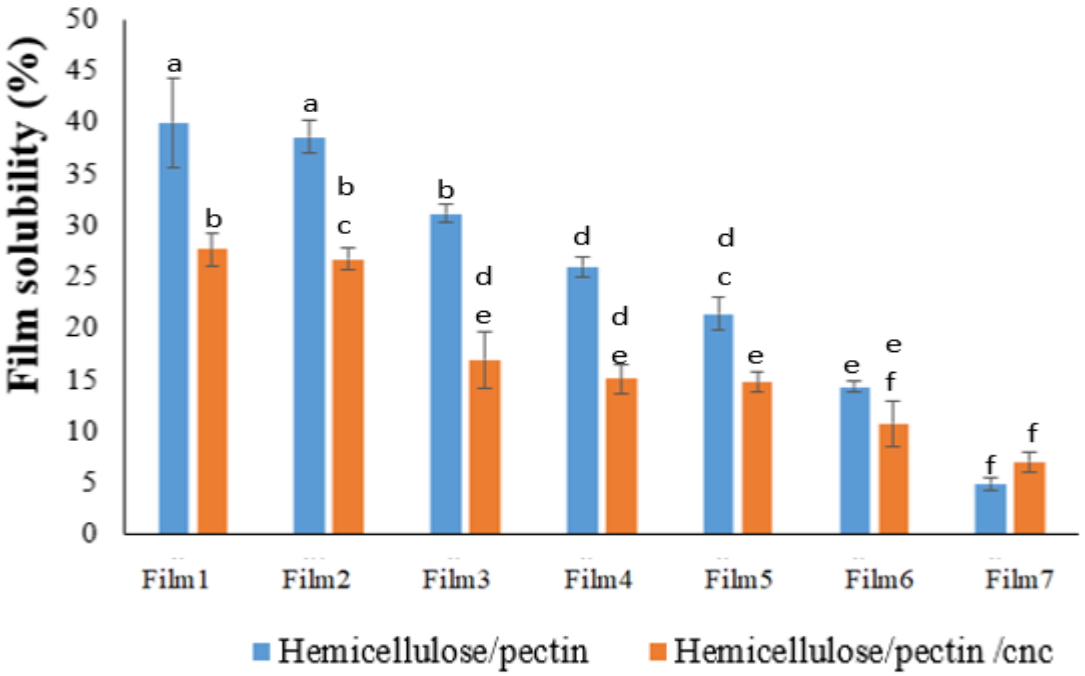
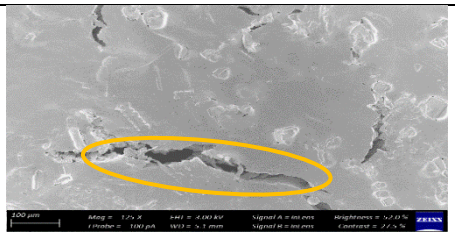
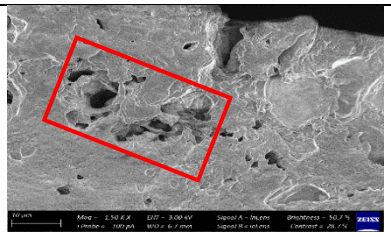
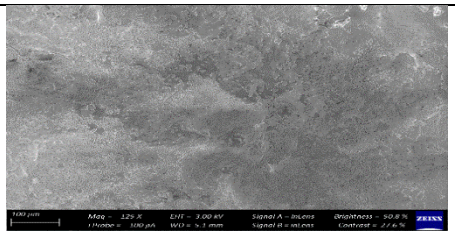
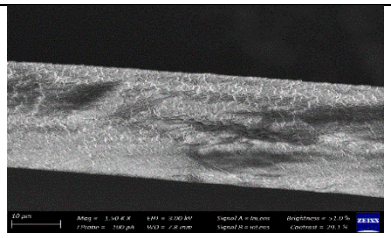
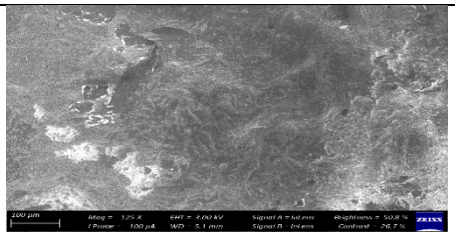
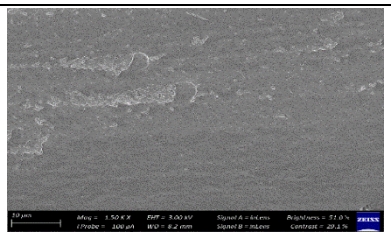
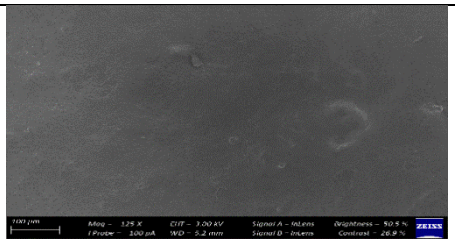
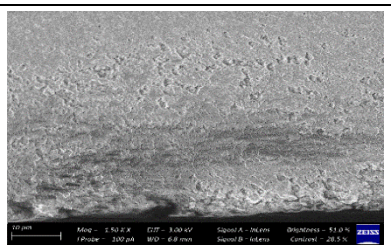
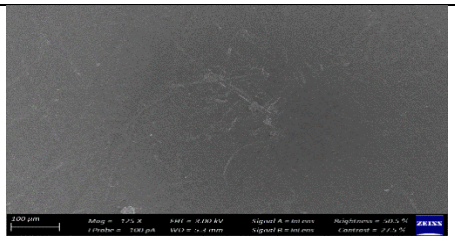
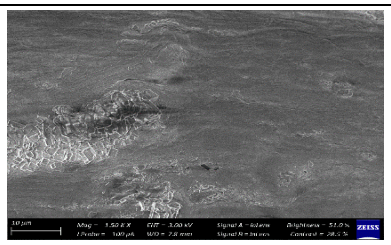
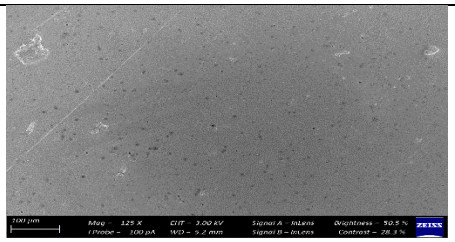
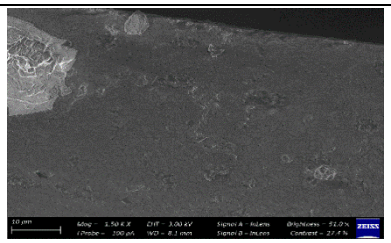


Figure 36: Hemicellulose-based films and pectin-based films solubility in the fatty food simulants, as affected by the ratio of hemicellulose to pectin. Hemicellulose: pectin ratios for bars in blue- Film 1: (5:0), Film 2: (4:1), Film 3 (3:2): Film 4: (2.5:2.5), Film 5: (2:3), Film 6: (1:4), Film 7: (0:5). Hemicellulose: pectin: nanocellulose (CNC) ratios for graph bars in orange- Film 1: (5:0:1.25), Film 2: (4:1:1.25), Film 3 (3:2:1.25): Film 4: (2.5:2.5:1.25), Film 5: (2:3:1.25), Film 6: (1:4:1.25), Film 7: (0:5:1.25). . The values are means of three measurements. Values with different letters are significantly different ($p < 0.05$). The error bars represent standard deviation

The integration of nanocellulose into pectin- and hemicellulose-based films further reduced the films' solubility in fatty food simulant, thereby increasing their potential application as packaging materials for fatty foods. Less of the hemicellulose-based films dissolved in the fatty food simulant (45% reduction) upon addition of 25% nanocellulose as a reinforcement. Further decline in solubility of hemicellulose-based films (up to 84%) was exhibited when both pectin and nanocellulose were added to the hemicellulose-based films (Figure 36) The reduction in dissolution of the biocomposite films could be because of the formation of hemicellulose-nanocellulose-pectin networks through hydrogen bonding (Fig. B2) (Chakrabaty and Teramoto, 2018). The surface profile images in Table 18 show voids in the hemicellulose film structure which are not seen when pectin and nanocellulose are incorporated into the hemicellulose films. Thus, the nanocellulose and pectin filled the voids in the hemicellulose structure which otherwise would act as channels for penetration of the simulant into the film (Kuorwel *et al.*, 2013; Hu *et al.*, 2016). The ethanol

accumulates within the film structure leading to swelling and breaking of the hydrogen bonds (Fortunati *et al.*, 2012).

Table 18: Surface and cross-section morphology of biocomposite films developed from hemicellulose, pectin and nanocellulose

Film type	Film Surface	Film cross-section
Hemicellulose[1]		
Pectin[2]		
Hemicellulose/pectin[3]		
Hemicellulose/CNC[4]		
Hemicellulose/pectin/CNC[5]		
Hemicellulose/pectin/CNC/ Polyphenol[6]		

Note: The circle shows cracks in the film and the rectangle shows the voids. Hemicellulose: pectin: nanocellulose ratio in film type 1: (5:0:0); type 2: (0:5:0); type 3: (3:2:0); type 4: (3.75:0:1.25); type 5: (3:2:1.25); type 6: (3:2:1.25). CNC: nanocellulose nanocrystals

Incorporation of 25% nanocellulose into the pectin films (Films 5 to 7) had no significant effect ($p > 0.05$) on the films' solubility in the fatty simulant, although a reduction in solubility of up to 31.1% was realised (Figure 36). The decrease in solubility can be attributed to the intermolecular bonding between the hydroxyl groups in the pectin and in the nanocellulose forming a barrier to the fatty simulant (Tongdeesoontorn *et al.*, 2011). However, pectin films with hemicellulose as a filler had their solubility reduced on addition of nanocellulose (Figure 36). This could be because hemicellulose introduces voids in pectin films, which are filled upon addition of the nanocellulose (Table 18). Therefore, pectin-based films require nanocellulose as a filler to reduce the solubility of the films in the fatty simulant.

6.5.2. Effect of hemicellulose/pectin ratio and nanocellulose reinforcement on the mechanical properties of biocomposite films

The pure hemicellulose films (Film 1) had lower tensile strength (Figure 37a) and Young's modulus (Figure 37b) when compared to pure pectin films (Film 7). The presence of cracks and voids (Table 18) in the hemicellulose structure could have contributed to the lower tensile strength and Young's modulus when compared to the intact structure of pectin films (Figure 37). However, these cracks and voids promoted flexibility in hemicellulose films, hence, the higher percent elongation of hemicellulose 478.64% when compared to that of pectin films (39%) (Figure 37c). The defects reduce intermolecular forces between hemicellulose polymer chains in the film allowing the chains to slide over each other when the film is stretched (Vieira, Da Silva, Dos Santos, & Beppu, 2011).

The tensile strength and Young's modulus of hemicellulose films improved when pectin was added as a filler (Figure 37a and b). Pectin, therefore, minimised the cracks and voids in the hemicellulose films, as can be seen in Figure 37. However, the pectin filler negatively affected the elasticity of hemicellulose films by up to 92.44% (Figure 37c). A reduction in hemicellulose flexibility (from 312.09% to 28.80%) was observed when pectin content in the hemicellulose-based films increased from 0-50% (Figure 37c). This could be due to the stiffness of pectin, denoted by a low percent elongation (19.08%) (Figure 37c). A further improvement in tensile strength and Young's modulus and decline in flexibility of hemicellulose-based films was observed when 25% w/w nanocellulose was added to the films (Figure 37). Nanocellulose has excellent reinforcing effects on biopolymers, hence, the improvement in Young's modulus in the pectin-based films (Ansari, Salajková, Zhou, & Berglund, 2015; Huang *et al.*, 2018). The further reduction in percent elongation of hemicellulose-based films by 34.8% with addition of 25% nanocellulose can be attributed to the stiffness of the nanocellulose fibres (Osoka & Onukwuli, 2018). The results recorded by Abdollahi, Alboofetileh, Rezaei, & Behrooz, (2013) showed a similar trend when alginate films were reinforced with nanocellulose (Abdollahi *et al.*, 2013).

On the other hand, adding hemicellulose as a filler (0-50%) to pectin films reduced the pectin-based films Young's modulus and tensile strength (Figure 37b). This could be because the hemicellulose filler disturbed the bonding network in the pectin films (Table 18), resulting in the formation of areas of stress in the film. Concentration of stress areas in the film weakens the film, hence, reducing the Young's modulus and tensile strength of the film (Manohar *et al*., 2015). In view of the porous structure displayed in hemicellulose films, adding hemicellulose to pectin films is likely to introduce voids (Table 18). Consequently, the presence of voids in pectin-based films may have resulted in higher elasticity in the films (by up to 42.2%) (Figure 37c) at the expense of the Young's modulus and tensile strength of the film.

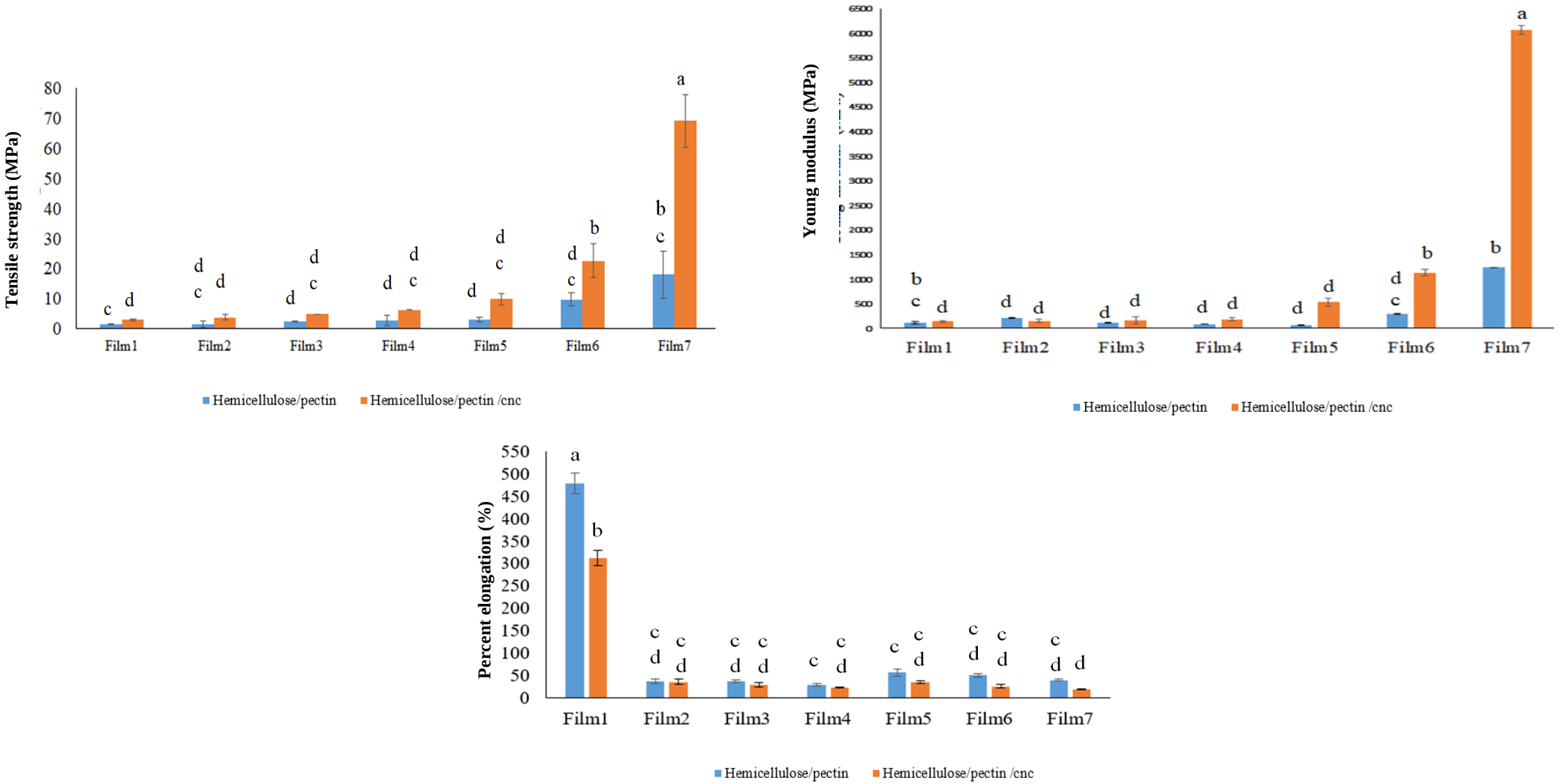


Figure 37: Hemicellulose-based films and pectin-based films mechanical properties, as affected by the ratio of hemicellulose to pectin. Hemicellulose: pectin ratios; Film 1: (5:0), Film 2: (4:1), Film 3: (3:2), Film 4: (2.5:2.5), Film 5: (2:3), Film 6: (1:4), Film 7: (0:5). CNC: nanocellulose crystals. The values are means of three measurements. Values with different letters are significantly different ($p < 0.05$). The error bars represent standard deviation

It is worth noting that adding 25% nanocellulose (by weight) and reducing the amount of hemicellulose filler (filler reduction from 67% to 0%) in pectin-based films, led to an increase in the tensile strength of the films by up to 284.9% (Figure 37a) and Young's modulus by up to 390.4% (Figure 37b). The increase in tensile strength and Young's modulus in pectin-based films and hemicellulose-based films when 25% nanocellulose was added could be due to the hydrogen bonding and ionic bonding occurring between hydroxyl groups in the nanocellulose and hemicellulose and hydroxyl and carboxyl groups in the pectin (Tongdeesoontorn *et al.*, 2011). Therefore, pectin and nanocellulose can be added as fillers to hemicellulose films to enhance the hemicellulose films mechanical properties. On the other hand, hemicellulose can be used as a plasticizer in pectin films. Kamdem *et al*. (2019) observed an increase in film elongation after adding hemicellulose to chitosan films.

6.5.3. Effects of hemicellulose/pectin ratio and nanocellulose reinforcement on the biocomposite structural properties

6.5.3.1. Interaction of components within the hemicellulose- and pectin-based films

The O-H peak (3300 cm^{-1} peak) for pure hemicellulose films (Figure 38a) was sharper than that of the pure pectin films (Figure 38b) and nanocellulose films (Figure 38c). It can be deducted from these results that hemicellulose has a higher hydroxyl group content than pectin and nanocellulose. The addition of nanocellulose and pectin to hemicellulose films (Figure 38d and e) resulted in a reduction in the stretching of the O-H bands, which is evidence of hydrogen bonding between the hemicellulose, pectin and nanocellulose. The relatively lower intensity of the stretching of the O-H groups (3300 cm^{-1} peak) in hemicellulose/nanocellulose films (Figure 38e) than in hemicellulose/pectin films (Figure 38d) could be an indication of the hemicellulose having a higher affinity for nanocellulose when compared to pectin. The differences in FTIR spectra of the hemicellulose/pectin/nanocellulose films without (Figure 38g) and with polyphenols (Figure 38h) shows that the polyphenols had an impact on the structure of the films. That is, the O-H peak in the hemicellulose/pectin/nanocellulose films diminished upon integrating polyphenols. Therefore, the polyphenols formed hydrogen bonds with the film components, thereby reducing the hydroxyl groups content of the films. Therefore, the structural properties of biocomposites can be manipulated by blending different biopolymers.

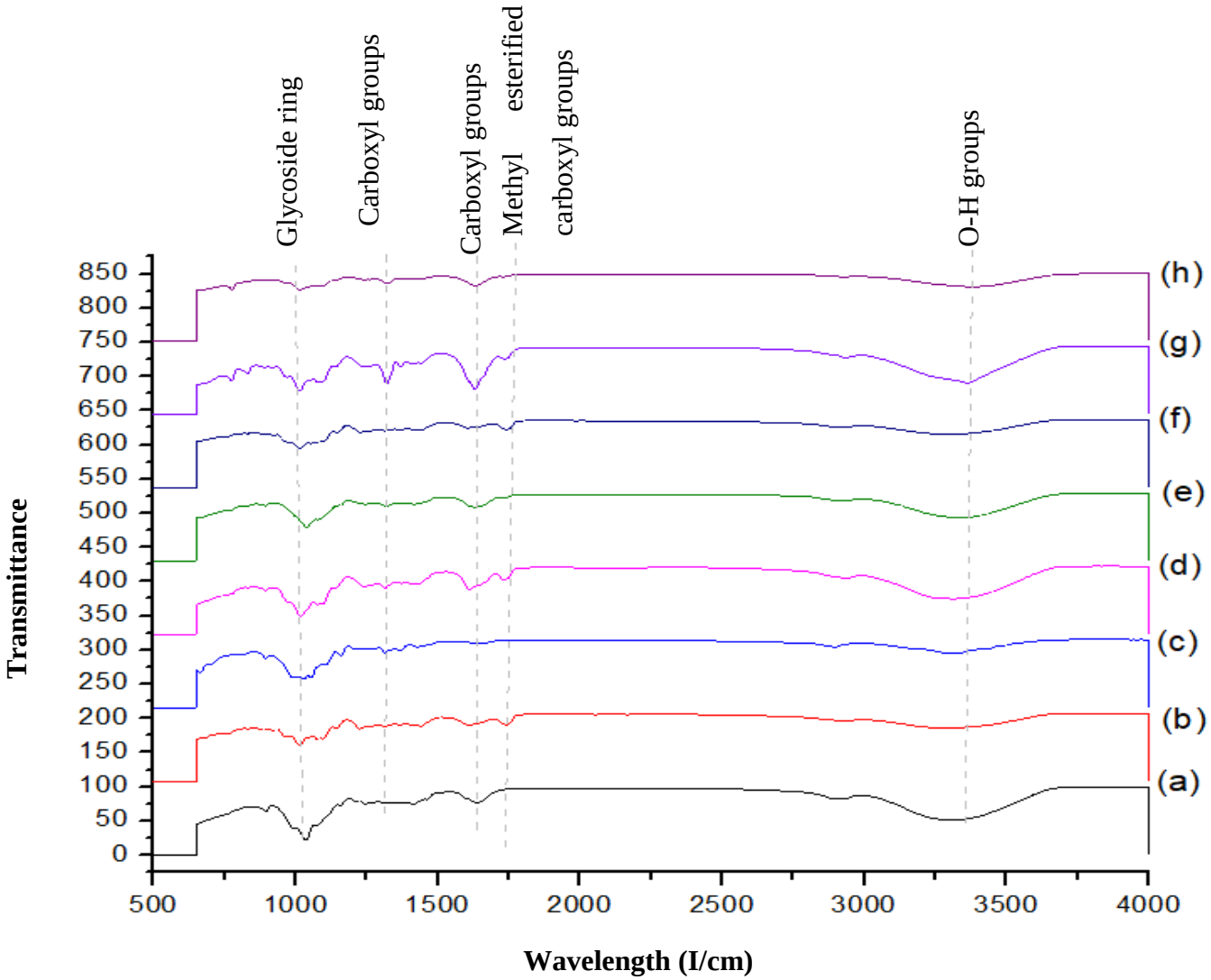


***Figure 38**: FTIR spectra of (a) hemicellulose film (b) pectin film (c) nanocellulose film (d) hemicellulose/pectin film (e) hemicellulose/nanocellulose film (f) pectin/hemicellulose film (g) hemicellulose/pectin/nanocellulose film (h)hemicellulose/pectin/nanocellulose/polyphenol film*

6.5.3.2. Surface profiles of hemicellulose/pectin/nanocellulose biocomposite films

Cracks and voids were observed on the film surface and in the cross-sectional area of the pure hemicellulose films (Table 18). These results support the fact that hemicellulose, on its own, cannot form films that can be applied for food packaging (Shimokawa *et al.*, 2015). The cracks form line of weakness in the packaging film, thereby facilitating film failure during usage (Yamak, 2016). Smoother films (films with no cracks or voids) were achieved when pectin and nanocellulose were added as fillers to the hemicellulose films (Table 18). On the other hand, pure pectin films and the pectin films with hemicellulose and nanocellulose as a filler had smooth surface profiles (Table 18). This is an indication that pectin films with or without fillers are suitable for food packaging application.

The surface profiles of the hemicellulose/pectin/nanocellulose films did not change upon addition of mango polyphenols (Table 18). The observed results are due to the relatively small particle size of the polyphenols (for example mangiferin, the major polyphenol type in the mango peel polyphenol extract has a molecular weight of 422.33 g/mol) and the capability of the polyphenols to form hydrogen bonds with the film components (Luo *et al.*, 2012; Renard *et al.*, 2017). Therefore, the hemicellulose/pectin/nanocellulose films were compatible with the mango polyphenols, which provides the basis for developing active packaging.

6.5.4. Hemicellulose/pectin/nanocellulose films potential application as active food packaging

6.5.4.1. Effect of temperature and time on polyphenol release from hemicellulose/pectin/ nanocellulose films

Biocomposite films made by blending hemicellulose, pectin and nanocellulose were capable of releasing mango peel polyphenols into the fatty food simulant in response to temperature and time (Figure 39). Both temperature and time had a significant effect ($p < 0.05$) on the migration of the polyphenols from the biocomposite into the fatty food simulant (Table 19). However, the interaction of these two factors had no effect on the polyphenol release into the simulant (Table 19). Therefore, temperature and time can be independently controlled to influence the release of the polyphenols. This aspect is important in maintaining food quality, as each factor (temperature and time) can negatively affect food quality even when the other factor is controlled. For example, food stored at optimum storage temperatures will still go bad after a certain period of time (Touati *et al.*, 2016). Hence, a packaging material capable of releasing polyphenols in response to the deviated factor will be the most ideal for packaging food.

Raising temperature from 5 ^{o}C to 40 ^{o}C and prolonging exposure time from 0 h to 48 h (Figure 39) boosted the release of polyphenols by the biocomposite (from 0% to 98.54%). The observed antioxidant release by the hemicellulose/pectin/nanocellulose films was higher than the reported release of thymol from polylactic acid (up to 60%) (Ramos *et al.*, 2014), however, it was lower than the release of tocopherols (100%) from the polylactic acid films (Jamshidian *et al.*, 2013). This shows that the rate of release of antioxidants from films is not dependent only on temperature and time but it is also affected by the type of film and antioxidant.

The rate of release of polyphenols by the biocomposite films into the fatty food simulant ranged from 0.014 mg/h to 0.021 mg/h (Figure B3). The results were within the range of release of potassium sorbate from pectin-methyl cellulose films (0.013 mg/h to 0.039 mg/h) into the fatty simulant with increase in temperature from 4 ^{o}C to 40 ^{o}C (Yu *et al.*, 2017). The least rate of polyphenol release was observed for the films incubated at 5 ^{o}C (0.014 ± 0.0004 mg/h) whilst the

highest rate of release was recorded for the films exposed to a temperature of 40 ^{o}C (0.021 ± 0.0017 mg/h). Incubating the films in contact with the simulant at higher temperatures (40 ^{o}C vs 5 ^{o}C) may have increased the vibration of the film molecules (Manzanarez-López *et al.*, 2011), thus increasing channels for the simulant to penetrate the film, dissolving the entrapped polyphenols, thereby boosting the rate of polyphenol release from the films. Additionally, the increase in rate of release (0.014 to 0.021 mg/h) of polyphenols by the films with increase in temperature and time could have been because of the increase in the ethanol-based hydrolysis of the bonding between the film and the polyphenols (Figure B2), resulting in polyphenol release (Samsudin *et al.*, 2014).

Considering that free radicals that promote food spoilage increase when temperature increases, a packaging material that is able to release antioxidants in response to temperature changes can, thereby increase food shelf life (Benbettaïeb *et al.*, 2018).

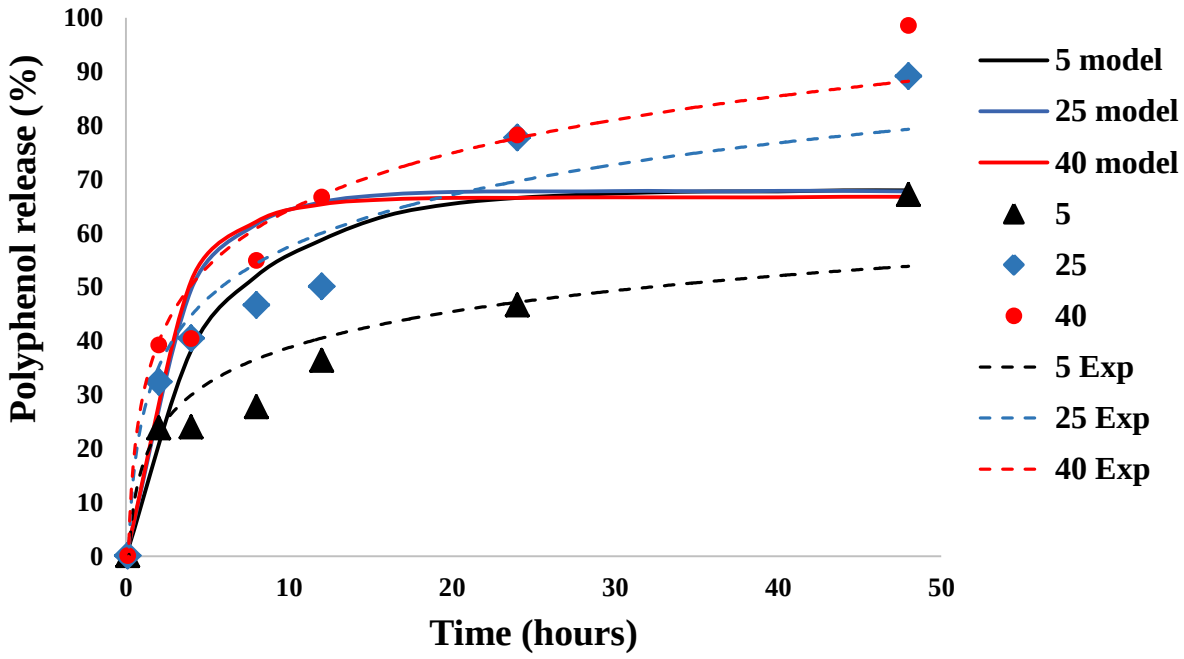


Figure 39: Hemicellulose/pectin/nanocellulose films polyphenol release into the fatty food simulant as affected by temperature. 5, 25, 40 represent temperatures in degrees Celsius, 5 model, 25 model and 40 model represent the temperatures in degrees Celsius at which polyphenol release was modelled. Dots are the experimental data. Exp represents experimental

The temperature dependent release of the polyphenols by the hemicellulose/pectin/nanocellulose films will ensure just in time addition of polyphenols to food, thereby preventing the accumulation of free radicals in food when food storage temperature increases (Bhunia *et al.*, 2013). It is worth noting that the release of polyphenols from the films, which ranged from 17.45 ± 0.451 mg/kg simulant to 25.61 ± 2.71 mg/kg (Figure B3), was below the allowable packaging films additives release limit of 60 mg/kg of simulant (EU, 2011). Therefore, hemicellulose/pectin/nanocellulose

films have potential application as active packaging material with controlled release of antioxidants into the fatty foods (storage temperatures ranging from 5 ^{o}C to 40 ^{o}C for 2 days).

The controlled release of mango polyphenols from hemicellulose/pectin/nanocellulose films proves that these films are compatible with the mango peel polyphenols, possibly because both the polyphenols and the hemicellulose/pectin/nanocellulose films contain hydroxyl groups (Farhat, Venditti, *et al.*, 2017). Hence, the hydrogen bonding of the films and the antioxidants prevent the instant release of the polyphenols by the films into the simulant (Tang *et al.*, 2003).

Table 19: Anova results for the effects of temperature and time on hemicellulose/pectin/nanocellulose film polyphenol release and simulant antioxidant activity

Dependent variable	P-Values		
	Time	Temperature	Time*Temperature
Polyphenol release	0.000	0.000	0.657
Antioxidant activity	0.077	0.017	0.433

6.5.4.2. Comparison of experimentally obtained polyphenol release by hemicellulose/pectin/nanocellulose films and model predictions

The experimental results for polyphenol release from hemicellulose/pectin/nanocellulose films had a logarithmic trend as shown in Figure 39. The hemicellulose/pectin/nanocellulose films exhibited a similar polyphenol release profile as that predicted by Migratest Exp (Figure 39). The mathematical trend fitting models for the experimental data are in the Appendix section (Table B3). Hence, the hemicellulose/pectin/nanocellulose films obey Fick's differential law of diffusion (Poças *et al.*, 2016). The migration models developed using Migratest Exp software were able to give accurate predictions of the hemicellulose/pectin/nanocellulose film release of mango polyphenols within the first 4 h of exposing the films to the simulant (Figure 39). However, the model predictions deviated more from the experimental results as temperature decreased from 40 ^{o}C to 5 ^{o}C and film contact time with the simulant increased from 4 h to 48 h. At 25 ^{o}C and 40 ^{o}C, the model underestimated the polyphenol release of the films while an overestimation of the polyphenol release was observed for the films at 5 ^{o}C (Figure 39). The deviation of the model predictions from the experimental results is because the models do not factor in the effect of the fatty food simulant and temperature on the structure of hemicellulose/pectin/nanocellulose film. For instance, raising temperature promotes the dissolution of the film in the simulant, making the film more porous, thus, increasing the rate of release of the polyphenols into the simulant (de Abreu *et al.*, 2009). Hence, at higher temperatures (25 and 40 ^{o}C) the Migratest Exp models underestimated the polyphenol release by the biocomposite films. It is, therefore, evident that the overestimation of polyphenol migration at 5 ^{o}C by the Migratest models is probably due to the lower solubility of

hemicellulose/pectin/nanocellulose films in the simulant at the low temperature. Researchers have recommended that migration modelling be applied for active food packaging design only when the models overestimate the release of the active compounds from the packaging material (Gavriil *et al.*, 2018; Stärker and Welle, 2019). This is to protect the consumer from over exposure to food packaging additives (Gavriil *et al.*, 2018). Therefore, in this case, migration models are best suitable for predicting the release of mango polyphenols for food stored at 5 ^{o}C for 2 days.

6.5.4.3. Effect of temperature and time on antioxidant activity of the fatty food simulant in contact with hemicellulose/pectin/nanocellulose films

Temperature and time had a significant effect ($p < 0.05$) on the antioxidant activity of the fatty food simulant in contact with the hemicellulose/pectin/nanocellulose/polyphenol film, but the interaction of the factors had no effect (Table 19) Similar results were obtained for the release of polyphenols by the films into the fatty food simulant (Table 19). Furthermore, the antioxidant activity of the fatty food simulant (Figure 40) was observed to increase with an increase in polyphenol release into the fatty simulant (Figure 39) by the films. Therefore, the polyphenol content of the food simulant contributed to the free radical scavenging capabilities of the fatty food simulant. The change in simulant antioxidant activity with time followed a logarithmic trend as can be seen in Figure 40. The trend fitting models are given in the Appendix (Table B4). The rapid release of polyphenols by the films during the first 10 h (Figure 39) resulted in a swift increase in the antioxidant activity of the simulant, while the release of the polyphenols after the first 10 h led to a slight increase in antioxidant activity. Therefore, the polyphenol content of the food simulant contributed to the free radical scavenging capabilities of the fatty food simulant.

The antioxidant activity of the fatty food simulant in contact with the hemicellulose/pectin/nanocellulose/polyphenol films incubated at 5 ^{o}C, 25 ^{o}C and 40 ^{o}C increased from 0% to 14.88%, 18.83% and 22.55%, respectively, with increase in exposure time to 48 h (Figure 40). The increase in antioxidant activity of the fatty simulant with increase in temperature can be due to the increase in rate of diffusion of antioxidants from the films (Figure 39) with increase in temperature (Koontz *et al.*, 2010). The ability of the hemicellulose/pectin/nanocellulose films doped with polyphenols to raise the antioxidant activity of the fatty food simulant in response to temperature and time further confirms the suitability of the films as active packaging of fatty foods. Reactions that boost the generation of free radicals which cause food spoilage are facilitated by a rise in temperature from 5 ^{o}C to 40 ^{o}C. Therefore, if the antioxidant activity of the food simulant is high at such conditions, the free radicals are minimised, thereby increasing food shelf life (Arrieta *et al.*, 2014).

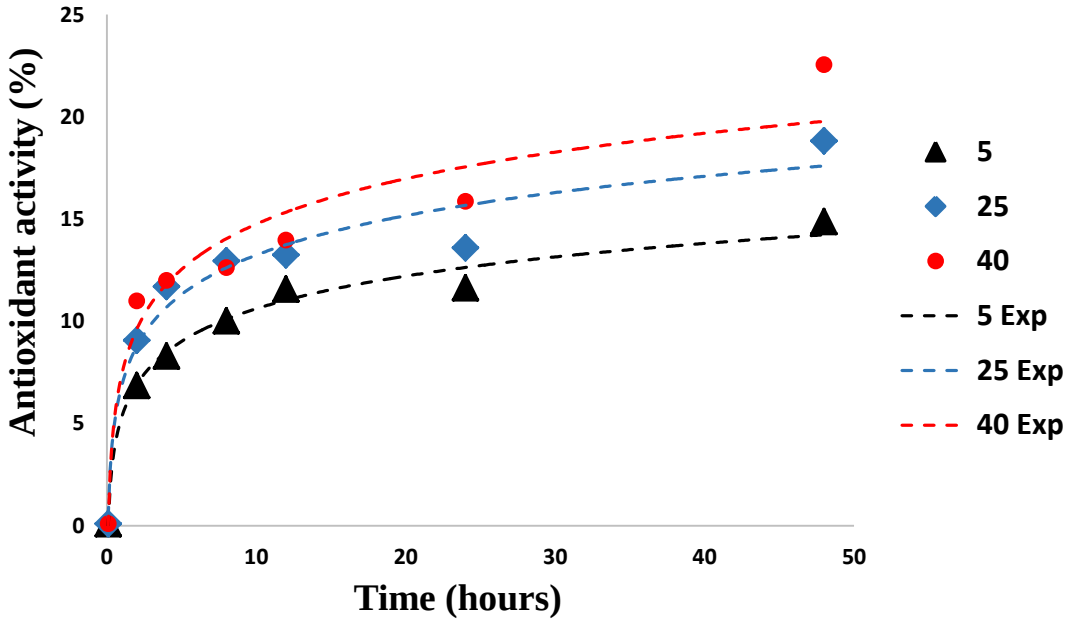


Figure 40: Antioxidant activity of the fatty food simulant as affected by temperature. 5, 25, 40 represent temperatures in degrees Celsius. Dots are the experimental data. Exp represents experimental

6.5.4.4. Thermal stability of biocomposites containing hemicellulose, pectin and nanocellulose

Hemicellulose films had more thermal stability than pectin films. Hemicellulose films had an initial degradation temperature ($T_{5\%}$) of 116 ^{o}C and that for pectin films was 89 ^{o}C (Figure 41). The thermal stability of hemicellulose-based films (hemicellulose/pectin films) was reduced upon adding pectin to hemicellulose films. This can be deduced from the reduction in $T_{5\%}$ value from 116 ^{o}C to 62 ^{o}C upon adding pectin to hemicellulose films. Incorporating nanocellulose and polyphenols in hemicellulose/pectin films enhanced the thermal stability of the films. Hemicellulose/pectin films (338 ^{o}C, 62 ^{o}C) had a lower T_{max} (temperatures for maximum weight loss) and $T_{5\%}$ than hemicellulose/pectin/nanocellulose films (347 ^{o}C, 86 ^{o}C) and hemicellulose/pectin/nanocellulose/polyphenol films (362 ^{o}C, 91 ^{o}C) (Figure 41). This could be due to the formation of strong interaction between the biopolymers and the polyphenols (Kamdem *et al.*, 2019). The higher thermal stability of the hemicellulose films with polyphenols than without confirms that the mango polyphenols are compatible with hemicellulose/pectin/nanocellulose films.

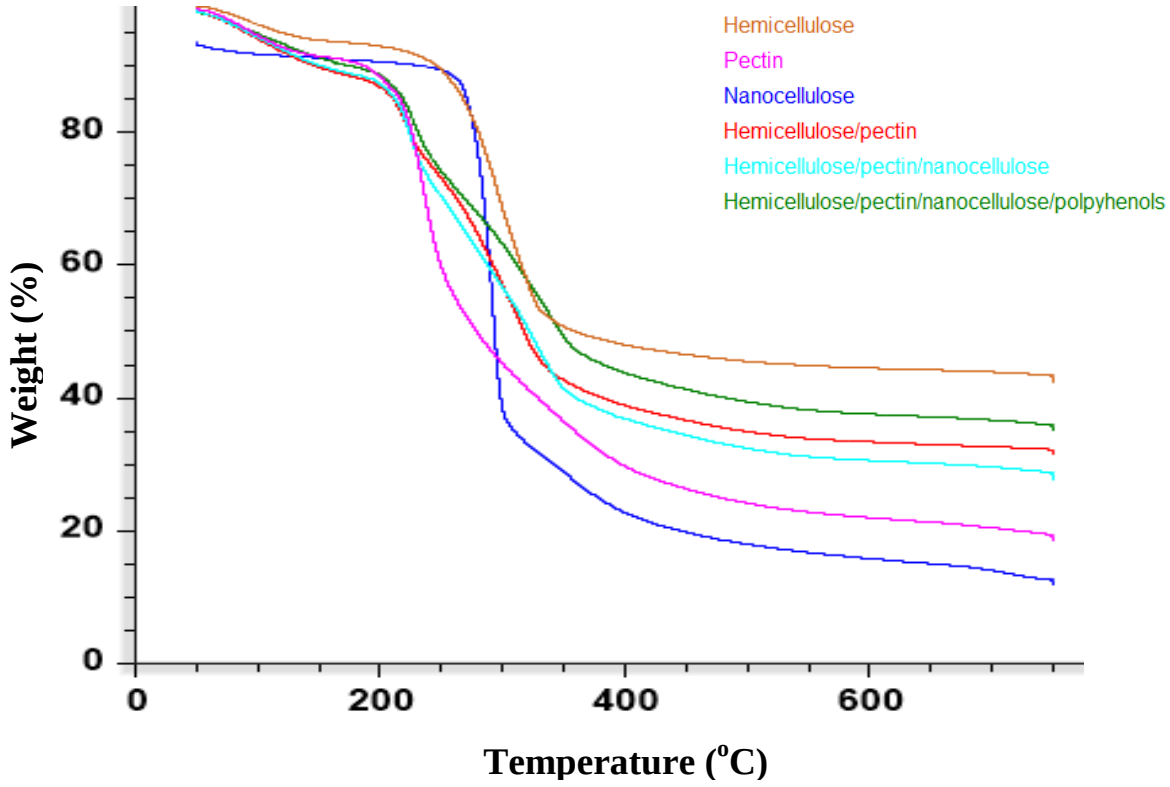


Figure 41: Thermogravimetric analysis of hemicellulose-based and pectin-based films

6.6. Conclusions

The ratio of hemicellulose and pectin in composites can be manipulated to produce films with different functionalities. Hemicellulose and pectin can be used as fillers in pectin-based films and hemicellulose-based films respectively depending on the desired functionality of the biocomposite. Thus, biocomposites with > 50% hemicellulose are suitable for application as active packaging films while the films with > 50% pectin are appropriate for application as edible films and coatings. Hemicellulose-based films' Young's modulus and tensile strength can be enhanced by adding pectin and nanocellulose as fillers. The two fillers minimise the dissolution of the hemicellulose-based films when in contact with the fatty simulant. A hemicellulose filler improves the flexibility of pectin-based films. Hemicellulose/pectin/nanocellulose films are capable of encapsulating and releasing bioactive substances as demonstrated by the encapsulation and release of polyphenols into the fatty simulant in response to variations in temperature and time, thus increasing their potential to be used as lipid barrier packaging with active properties for increasing food shelf life. Migration models based on Fick's law of diffusion can be used for predicting polyphenol release from hemicellulose/pectin/nanocellulose films

6.7. Paper Title: "Reinforcement efficiency of acetylated nanocellulose as a filler in acetylated hemicellulose films"

Prepared for submission to the journal of Composites Part B: Engineering (impact factor 7.635)

6.8. Abstract

The study investigated the effects of varying acetylated nanocellulose (ACNC) degree of substitution (DS) from 0-2.34 and loading from 10-50% on the mechanical properties (Young's modulus and tensile strength) of acetylated hemicellulose-based films (AH). Micromechanical models were applied to predict the AH mechanical properties and to estimate nanocellulose reinforcing efficiency. Young's modulus prediction was carried out using the Halpin-Tsai, isostrain, isostress, Tsai-Pagano, and Cox-Krenchel models while Bowyer-Bader, Modified Rule of mixtures, Hirsh, parallel and series models were used to predict the tensile strength of the AH/ACNC biocomposite films. The variation of the nanocellulose acetylation from DS 0 to 2.34 improved the AH Young's modulus by 59.2% and tensile strength by 101.2%. Increasing the ACNC loading from 10-50% improved the reinforcing efficiency of the ACNC in AH based films from 0.059 to 6.29 with Young's modulus increasing from 63.68 to 590.15 MPa and tensile strength from 2.28 MPa to 10.59 MPa. The tensile strength and Young's modulus were best predicted (95% confidence level) with the Bowyer-Bader and the Cox-Krenchel models, respectively. Therefore, nanocellulose acetylation enhances its compatibility with AH. The development of AH films, with varying mechanical properties can be predicted before film development, which allows film customisation for various applications.

Keywords: Acetylated hemicellulose; Acetylated nanocellulose; Theoretical models; Mechanical properties; Acetylation,

6.9. Introduction

Hemicellulose has recently received attention as an alternative raw material for food packaging material because of its lightweight, biodegradability and abundance (Shimokawa *et al.*, 2015; Huang *et al.*, 2018; Li and Pan, 2018). However, the poor mechanical properties of pure hemicellulose films emanating from hemicellulose hydrophilicity limits its application as a replacement for synthetic food packaging material (Farhat, Venditti, *et al.*, 2017; Li and Pan, 2018). In order to curb these drawbacks, the hemicellulose structure is chemically modified by processes such as acetylation, grafting and silane coupling (Belmokaddem *et al.*, 2011; Gordobil *et al.*, 2014) and/or reinforcing with a filler such as nanoclay, nanocellulose, chitosan and montmorillonite (Junli *et al.*, 2012; Chen *et al.*, 2015; Chen *et al.*, 2016; Liu *et al.*, 2016).

Many researchers have reported that the most suitable modification method for the hemicellulose used in the formulation of food packaging material is acetylation (Xu *et al.*, 2010; Stepan *et al.*, 2012; Szabo *et al.*, 2015; Farhat, R. Venditti, *et al.*, 2017). Acetylation involves replacing hydrogen atoms in hydroxyl groups in a polymer structure with acetyl groups rendering the polymer less hydrophilic (Yagyu *et al.*, 2017). Acetylation yields a substantial improvement in the Young's modulus from 3 MPa to 2300 MPa and tensile strength from 3.3 MPa to 44.1 MPa when compared to unmodified hemicellulose (Gordobil *et al.*, 2014).

Further improvements in mechanical properties, are achieved by reinforcing the modified hemicellulose films with hydrophobic fillers. Among the aforementioned fillers, nanocellulose is the most attractive reinforcing material for hemicellulose films because of its excellent mechanical properties, namely, Young's modulus ranging from 100-140 GPa and tensile strength ranging from 8-10 GPa (Ioelovich, 2017; Kassab *et al.*, 2019) and ease to modification by acetylation (Chakrabaty and Teramoto, 2018). Acetylation reduces self-agglomeration of nanocellulose, enhancing its dispersion and interaction with hydrophobic polymers, thereby improving the mechanical properties of the reinforced matrix (Zhang *et al.*, 2016). The major contributing factors to ACNC distribution and bonding within a matrix are ACNC DS and loading (Lepetit *et al.*, 2017). Therefore, nanocellulose acetylation DS and loading in a composite matrix need to be considered for effective reinforcing.

Hemicellulose has been acetylated to a DS of 1.8 and reinforced with cellulose and nanocellulose with DS of 0 and 0.54 respectively, however, the effects of increasing the DS beyond 0.54 and ACNC loading on AH films mechanical properties, have not been studied (Gordobil *et al.*, 2014). Trends of ACNC DS and ACNC loading against mechanical properties have however, been studied for PCL, and LDPE and these studies have shown that increasing ACNC DS did not cause a change in the mechanical properties of LDPE, however, it resulted in improved mechanical properties of

PCL by up to 70% (Yang *et al.*, 2010; Lepetit *et al.*, 2017). The limiting factor for adopting these trends in AH is that, unlike PCL and LDPE, which are completely hydrophobic, whereas, AH (DS 1.7) contains hydrophilic groups which may affect its interaction with ACNC. Nevertheless, there is potential to increase the mechanical properties of AH through addition of ACNC with a DS higher than 0.54. The improvement in these properties is attributed to increased hydrophobic interactions between the AH and ACNC with an increase in acetyl groups (Yang *et al.*, 2010).

Furthermore, an understanding of the micromechanical interactions (reinforcing efficiency) of the reinforcing ACNC in the AH is important for enhancing biocomposite mechanical properties during biocomposite design. The formation of bonds between the matrix and the reinforcing material results in a matrix-reinforcing material network capable of withstanding stress and strain, whereas if no bonding occurs, voids may form in the matrix (Lee *et al.*, 2014; Osoka and Onukwuli, 2018). The voids are areas of weakness in the composite which reduce the mechanical properties of composites, thereby lowering the reinforcing efficiency of the reinforcing material (Tham *et al.*, 2019). Reinforcing efficiency is a ratio, obtained using Equation 24, highlighting the contribution of a reinforcing fibre to the mechanical properties of a composite. The reinforcing fibre parameters considered when calculating reinforcing efficiency are the elastic modulus of the fibre and the fibre loading (Osoka and Onukwuli, 2018). The reinforcing efficiency of nanocellulose in different matrices such as polylactic acid, polypropylene, starch and chitosan has been derived from theoretical models such as the Halpin-Tsai models (Lin *et al.*, 2011; Lee *et al.*, 2014; Yaghoobi and Fereidoon, 2018). Nanocellulose had different impact on the aforementioned matrices, depending on the matrix-nanocellulose interaction (measured based on Young's modulus and tensile strength of composite), nanocellulose dimensions (length to diameter aspect orientation), orientation, distribution in the matrix and percentage filler loading. Aitomäki and Oksman, (2014)] categorised the reinforcing efficiency of fibres in polymer matrices into three classes namely, low, with a reinforcing efficiency less than 1, medium, with a reinforcing efficiency between 1 and 2.5 and high, with a reinforcing efficiency higher than 2.5. The reinforcing efficiency of nanocellulose in hemicellulose cannot be assumed based on the nanocellulose interaction with other composites because of the differences in chemical composition and compatibility with nanocellulose. Additionally, nanocellulose should be utilised as a reinforcing material in polymers where its reinforcing efficiency is high to ensure an effective utilisation of the natural fibre. Hence, it is essential to study the reinforcing efficiency of ACNC in AH in order to determine the optimum ACNC loading in AH (Zainudin *et al.*, 2014).

Over the years several theoretical models have been developed and used to predict the mechanical properties of composites (Jonoobi *et al.*, 2010; Andre *et al.*, 2016; Triyono *et al.*, 2017).

The use of theoretical models is a cost and time effective method for predicting the mechanical performance of a composite prior to its manufacture (Andre *et al.*, 2016). Previous researchers have used these theoretical models to predict the Young's modulus, and tensile strength of both synthetic and biopolymer matrices reinforced with natural fibres (Delgado-Aguilar *et al.*, 2017). A micromechanical model that gives predictions within a 95% confidence interval of the experimentally obtained mechanical properties is considered the best fitting model (Osoka and Onukwuli, 2018). The performance of theoretical models in predicting mechanical properties is dependent on the biocomposite constituency and method of biocomposite production. For instance, the Young's modulus of the epoxy composites reinforced with kenaf fibre is best predicted with a Tsai-Pagano model, but the Young's modulus of unsaturated polyester reinforced with the same fibre is best estimated with a Halpin Tsai model. Similarly, the tensile strength of the epoxy composites and unsaturated polyester composites are predicted by different models, namely the Bowyer-Bader model and the Kelly Tyson model respectively (Du *et al.*, 2010; Andre *et al.*, 2016).

The commonly used theoretical models for predicting the Young's modulus of composites are Cox-Krenchel, Tsai-Pagano, Halpin-Tsai and for tensile strength are the Hirsch, Bowyer-Bader and the Modified rule of mixtures (MROM) (Zainudin *et al.*, 2014). The Cox-Krenchel model incorporates fibre diameter and length, matrix Poisson ratio, fibre distribution and orientation in predicting the Young's modulus of fibre reinforced biocomposite films (Raharjo *et al.*, 2018). The Cox-Krenchel model has the advantage of involving simple mathematical calculations and requires easily attainable data (Andre *et al.*, 2016). Unlike all the other models for Young's modulus estimation mentioned above, the Tsai-Pagano model takes into consideration the longitudinal and transverse modulus of the reinforcing fibres. The Tsai-Pagano model was developed on the basis that natural reinforcing fibres are complex and it may not be ideal to assume that these fibres have the same elasticity when stretched in different directions (Andre *et al.*, 2016). On the other hand, Halpin-Tsai models integrate reinforcing fibre length to diameter aspect ratio, orientation and the Young's modulus of the unreinforced matrix in determining the resultant reinforced biocomposite Young's modulus (Zainudin *et al.*, 2014).

The Hirsch model was derived from the series and parallel models, and does not take into consideration the fibre structure such as diameter and length when compared to the other tensile strength models (Delgado-Aguilar *et al.*, 2017; Raharjo *et al.*, 2018). The model only considers the stress transfer between the reinforcing fibre and the matrix and the fibre orientation (Raharjo *et al.*, 2018). Delgado-Aguilar *et al.*, (2017) highlighted that the Hirsch model requires data that can easily be obtained, making the model more favourable for industrial use than the other models which require fibre morphology data obtained using specialised equipment (Delgado-Aguilar *et al.*, 2017;

Espinach *et al.*, 2018). The Bowyer-Bader model stipulates that the tensile strength of a reinforced biocomposite is a sum of the tensile strength of the reinforced matrix and fibres (both the supercritical and subcritical fibres) (Andre *et al.*, 2016). Therefore, due to the different assumptions which were made in developing the micromechanical models, it is necessary to study several models to determine the most suitable model for predicting the mechanical properties of AH/ACNC composites.

The aim of this study was to investigate the effects of nanocellulose DS and loading on the mechanical properties of AH composites. In addition, the potential for using theoretical models to predict tensile strength and Young's modulus of AH reinforced with ACNC, was assessed. The reinforcing efficiency of the ACNC in the AH matrix was determined as a step towards determining the optimum ACNC loading in AH composites.

6.10. Materials and methods

6.10.1. Materials

The hemicellulose used in this study was extracted from wheat straw in our laboratory. Nanocellulose (nanocellulose crystals, freeze dried) was sourced from the University of Maine, Orono, U.S.A. Kimix Chemical & Lab Supplies (Cape Town, South Africa) supplied the following chemicals; NaOH , HCl, ethanol, sulphuric acid and phenolphthalein indicator. Sigma Aldrich, Germany, supplied DMSO, acetic anhydride and acetic acid. All the chemicals used in the experiments were of the analytical grade.

6.10.2. Methods

6.10.2.1. Experimental method

6.10.2.1.1. Hemicellulose acetylation

Hemicellulose was acetylated to a DS of 1.7 based on a method developed by Belmokaddem *et al.*, (2011). Hemicellulose was reacted with 2.5 mL of 99% acetic acid in a water bath maintained at 50^{o}C for 5 min. The reaction was carried out in Schott bottles. The mixture was then cooled in an ice bath to 25 ^{o}C prior to acetylation with 6.6 mL of 99% acetic anhydride and 200 µL of 98% sulphuric acid as a catalyst in a shaking water bath set at 50 ^{o}C for 1 h. Precipitation of AH was carried out with 150 mL of 95% ethanol prior to washing the AH four times with 100 mL of 95% ethanol. AH was oven dried at 40 ^{o}C for 24 h to a moisture content of less than 10%.

6.10.2.1.2. Nanocellulose acetylation

Nanocellulose was acetylated to a different DS according to the method used by Sahlin, (2015) with modification-acetylation temperature of 25 ^{o}C and 1 µL of sulphuric acid was used instead

of 40 ^{o}C and 200 µL respectively applied in the original method. The nanocellulose sample, 300 mg, was thoroughly mixed for 1 min with 3 mL of 99% acetic acid in 50 mL falcon tubes. Approximately 1 mL of 99% acetic anhydride and 1 µL of 98% sulphuric acid was added to the nanocellulose slurry. Nanocellulose of a different DS was obtained by varying the acetylation time from 10 min to 240 min maintaining the incubation temperature at 25 ^{o}C in a water bath. The acetylation process was halted through the addition of 150 mL water. The addition of water precipitated the acetylated nanocellulose. ACNC was washed three times with 150 mL of water followed by oven drying at 40 ^{o}C for 24 h to a moisture content of less than 10%.

6.10.2.1.3. Determination of acetylated hemicellulose and nanocellulose degree of acetylation

The acetylation degree of substitution of hemicellulose and nanocellulose was determined according to the method described in section 5.4.8

6.10.2.1.4. Development of acetylated hemicellulose/nanocellulose films

The AH/ACNC films were prepared using the solvent casting method based on the method by Egüés, Stepan, Eceiza, & Toriz, (2014) with modifications; Dimethyl sulfoxide was used as the solvent for film formation instead of chloroform which was used in the original method. In order to determine the trend in AH films mechanical properties with increasing DS (0-2.34), AH films reinforced with ACNC of varying DS were formed by dissolving 375 mg of AH and 125 mg of ACNC (25% w/w of total film solid content) in 20 mL of DMSO. For AH films with varying ACNC loading (DS 2.34), ACNC quantities of 10 to 50 wt. % of the total film solid content (500 mg) were dissolved in 20 mL of DMSO. The AH and ACNC were dissolved in DMSO by heating the mixture for 6 h at 50 ^{o}C in a shaking water bath. Films were formed by oven drying the homogenous slurry in 80 mm glass Petri dishes for two days at 50 ^{o}C. A 456CFSS elcometer was used to measure film thickness. Films with a thickness ranging from 64.125 to 83.6 µm were formed.

6.10.2.1.5. Determination of biocomposite mechanical properties

The mechanical properties, namely the tensile strength, Young's modulus and elongation of the films were tested with a model LRX instron tensile tester supplied by LLOYD instruments. The equipment was set at a crosshead speed of 50 mm/min. The gauge length of the tensile tester was maintained at 27.3 cm. Hemicellulose films strips cut to a length 6 cm and width of 1 cm were used for analysis.

6.10.2.2. Theoretical modelling

1. Reinforcement efficiency determination

Reinforcement efficiency of ACNC in AH was calculated based on Equation 24 below (Osoka and Onukwuli, 2018):

$$\xi = \frac{E_f(E_c - E_m) - V_f\, E_c(\, E_f - E_m)}{E_m[(E_f - E_c) - V_m(E_f - E_m)]} \tag{24}$$

where ξ is the reinforcement efficiency or modulus efficiency, E is elastic modulus and V is reinforcing fibre volume fraction, with the subscript c, m, f denoting composite, reinforced matrix and reinforcing fibre respectively. E_f was set at 138 GPa (Lee *et al.*, 2014), E_c and E_m were experimentally determined.

The reinforcing fibre volume fraction was calculated using the following equation:

$$V_f = \frac{\left(\frac{W_f}{\rho_f}\right)}{\left(\frac{W_f}{\rho_f}\right) + \frac{(1 - W_f)}{\rho_m}} \tag{25}$$

where the AH density, ρ_m =0.9 and ACNC density, ρ_f= 0.67, both densities were experimentally obtained. W_f is the weight percent of reinforcing fibre added to the composite.

2. Strength efficiency determination

Strength efficiency was calculated according to Lee *et al.* (2014). The strength efficiency calculation is based on comparing the experimental tensile strength to the theoretical tensile strength using the Equation (26) below:

$$Strength\ efficiency\ (\%) = \frac{Experimental\ tensile\ strength}{Theoretical\ tensile\ strength} \times 100\ \% \tag{26}$$

The theoretical tensile strength predicted by the Cox-Krenchel model (Equation 33) was used in this calculation.

3. Isostrain model

The isostrain model, Equation 27, also known as the parallel model assumes that the reinforcing fibres are continuous in the matrix. Both the matrix and the reinforcing material are presumed to be exposed to the same strain (Chen, 2015).

$$E_c = V_f E_f - V_m\, E_m \tag{27}$$

The longitudinal elastic modulus, E_f was set at 105 GPa (Ansari *et al.*, 2015), E_m = 55 MPa was experimentally determined, E_c is the model predicted Young's modulus, V_f was calculated using Equation 30. V_m was determined based on Equation (28) below:

$$V_m = 1 - V_f \tag{28}$$

4. Isostress model

The isostress model, Equation 29, also known as the series model assumes that the reinforcing fibre and reinforced material experience an equal stress. The model is based on the assumption that the reinforcing material is a continuous fibre and the tensile load is perpendicular to the fibres (Goel *et al.*, 2008). The isostress model equation is highlighted below:

$$E_c = \frac{E_f E_m}{E_m V_f + E_f V_m} \tag{29}$$

The transverse elastic modulus, E_f was set at 15 GPa (Ansari *et al.*, 2015). V_f and V_m were calculated using Equation 25 and 28 respectively.

5. Halpin-Tsai model

Halpin-Tsai model, Equation 30, predict the Young's modulus of composites with randomly dispersed reinforcing material. They take into consideration the geometry and quantity of the reinforcing material (Kundalwal, 2018). The Halpin-Tsai model equation is as follows:

$$E_c = E_m \frac{1 + \zeta \eta V_f}{1 - \eta V_f} \tag{30}$$

where ζ is the reinforcement material aspect ratio, = $2(\frac{\text{Length}}{\text{diameter}})$, and E_f was the elastic modulus of reinforcing material (138 GPa) (Lee *et al.*, 2014), V_f was calculated using Equation 25 and η is the efficiency factor. The efficiency factor is calculated from the Equation 31 below:

$$\eta = \frac{\left[\left(\frac{E_f}{E_m}\right) - 1\right]}{\left[\left(\frac{E_f}{E_m}\right) + \zeta\right]} \tag{31}$$

6. Tsai-Pagano model

The Tsai-Pagano model (Delgado-Aguilar *et al.*, 2017) is expressed as follows:

$$E_c = \frac{3}{8} E_{11} - \frac{5}{8} E_{22} \tag{32}$$

where E_{11} and E_{22} are the longitudinal Young's modulus and transverse modulus respectively. E_{11} and E_{22} are derived from the Halpin-Tsai model after setting the reinforcing aspect ratio (ζ) as

$2(\frac{\text{Length}}{\text{diameter}})$ for longitudinal Young's modulus and at ζ.=2 for the transverse modulus (Osoka and Onukwuli, 2018).

7. Cox-Krenchel model

The Cox-Krenchel model, Equation 33, are micromechanical models used to predict the Young's modulus of composites taking into consideration the reinforcing fibre dimensions and orientation in the fibres in the matrix (Raharjo *et al.*, 2018). In this study the fibre orientation is assumed to be random.

$$E_c = \eta_l \eta_o V_f E_f + V_m E_m \tag{33}$$

where $\boldsymbol{\eta_l}$ is the reinforcing fibre length factor, $\boldsymbol{\eta_o}$ is the orientation factor. The orientation factor is 0.375 for randomly orientated fibres (Andre *et al.*, 2016).

The fibre length factor was calculated using Equation 34 below.

$$\eta_l = 1 - \frac{\tanh(\frac{\beta l}{2})}{\frac{\beta l}{2}} \tag{34}$$

where β, coefficient for the rate of stress concentration at fibre ends, is given by

$$\beta = \frac{2}{d}\sqrt{\frac{E_m}{E_f(1-v_M)Ln(\frac{\pi}{x_i V_f})}} \tag{35}$$

x_i is the coefficient for the type of packing of the fibres in the composite. In this study, the value was set as 3.83 assuming random packing of the fibres in the matrix (Goel *et al.*, 2008). v_M is the Poisson ratio of the matrix, it was set as 0.33 (Marklund and Varna, 2009). d is the diameter of the reinforcing fibre, in this case it was 128.5 nm (experimentally determined).

8. Hirsch model

The Hirsch model, Equation 36, was derived from both the isostrain and isostress models taking into account both the longitudinal tensile strength and transverse tensile strength to calculate the overall tensile strength of reinforced composite (Delgado-Aguilar *et al.*, 2017).

$$T_c = x(V_f T_f + V_m T_m) + (1-x)\frac{T_f T_m}{T_m V_f + T_f V_m} \tag{36}$$

where T_c, T_f, T_m are the tensile strength of composite, fibre and matrix respectively. x is a coefficient of stress transfer between the fibre and the matrix x was set at 0.1 which is the coefficient value of stress transfer for randomly orientated fibres (Munde and Ingle, 2015).

9. Bowyer-Bader model

The Bowyer-Bader model, Equation 37, takes into account both the fibre dimensions and how they are arranged in the matrix when calculating the tensile strength of composites. The model equation is highlighted below:

$$T_c = \mathrm{T}_f \mathrm{K}_1 K_2 V_f + T_m\, V_m \tag{37}$$

where T_c, T_f, T_m are the tensile strength of composite, fibre and matrix respectively. K_1 and K_2 are reinforcing fibre orientation factor and fibre length factor respectively. In order to calculate fibre length factor two equations can be used depending on whether the length of the fibre is greater or less than the critical fibre length. For length greater than the critical length Equation 38 is used:

$$l > l_c, K_2 = \frac{l - l_c}{2l} \tag{38}$$

For length smaller than the critical length the Equation 39 below is used to calculate the length factor.

$$l < l_c, K_2 = \frac{l}{2l_c} \tag{39}$$

Where l is the length of the reinforcing fibre and l_c is the critical length denoted by

$$l_c = \frac{d \times T_f}{2\tau_i} \tag{40}$$

And τ_i the shear strength of the matrix is estimated using the following equation:

$$\tau_i = \frac{T_f}{\sqrt{3}} \tag{41}$$

where d is the fibre diameter, T_f is determined by Equation 42:

$$T_f = \frac{E_f \times T_c}{E_c} \tag{42}$$

10. Modified rule of mixtures (MROM)

The MROM model equation is as follows (Lee, Aitomäki, *et al.*, 2014):

$$T_c = \mathrm{T}_f \mathrm{K}_1 K_2 V_f + T_{mf}(1 - V_f) \tag{43}$$

where T_{mf} is the reinforced composite tensile strength, given by

$$T_{mf} = \frac{E_m \times T_f}{E_f} \tag{44}$$

where K_1 was set at 0.2 as the orientation factor for randomly orientated fibres (Lee, Aitomäki, *et al.*, 2014) an K_2 was calculated using Equation 39.

6.10.3. Statistical analysis

Experiments were done in triplicate. The Duncan's multiple range test was performed in Statistica 7 version 13.2 for means comparison.

6.11. Results and discussions

6.11.1. Effect of degree of substitution of acetylated nanocellulose on mechanical properties of acetylated hemicellulose

AH films reinforced with ACNC of DS 0.56 and 1.6 showed weakened mechanical properties, that is lower Young's modulus and tensile strength, when compared to the AH/ACNC films reinforced with unmodified nanocellulose (Figure 42). The phenomenon could be attributed to the reduction in hydrogen bonding between the AH matrix and the ACNC filler, (Dong *et al.*, 2017) compared to the hydrogen bonding that occurs in an AH-CNC (unmodified nanocellulose) biocomposite. As the acetylation DS of the CNC increased from 0 to 2.34, the number of available hydroxyl groups for hydrogen bonding with hydroxyl groups present in AH (DS1.7) diminished (Akkus *et al.*, 2018). A gradual increase in mechanical properties was observed as the ACNC DS increased beyond 1.6 to 2.34 (Figure 42). At a DS of 2.34 the ACNC had enhanced hydrophobicity, which increased the hydrophobic interactions between the ACNC and AH, an effect that has been reported in previous studies (Fortunati *et al.*, 2012). The mechanical properties of the composite films reinforced with the ACNC with DS of 2.34 significantly exceeded ($p < 0.05$) those of the ACNC with DS of 1.6 and below. Therefore, there is a minimum DS for enhancing such hydrophobic interactions between AH and ACNC in AH/ACNC biocomposite films. The effect can be attributed to the presence of a higher content of acetyl groups in films containing hemicellulose and nanocellulose at higher DS. The interaction between the hydrophobic groups is stronger than hydrogen bonds emanating from the presence of hydroxyl groups in the films (Gordobil *et al.*, 2014).

A higher acetyl DS in acetylated polymers increases dispersibility (Dong *et al.*, 2017), which promotes effective interaction between the two polymers due to reduced self-agglomeration of the polymers. In addition, acetylation reduces polarity of the nanocellulose, thereby increasing the repulsive forces among the nanocellulose chains and promoting dispersion of the nanocellulose in the hemicellulose films (Ahmadi *et al.*, 2018). Therefore, nanocellulose acetylation DS had a positive impact on film mechanical properties

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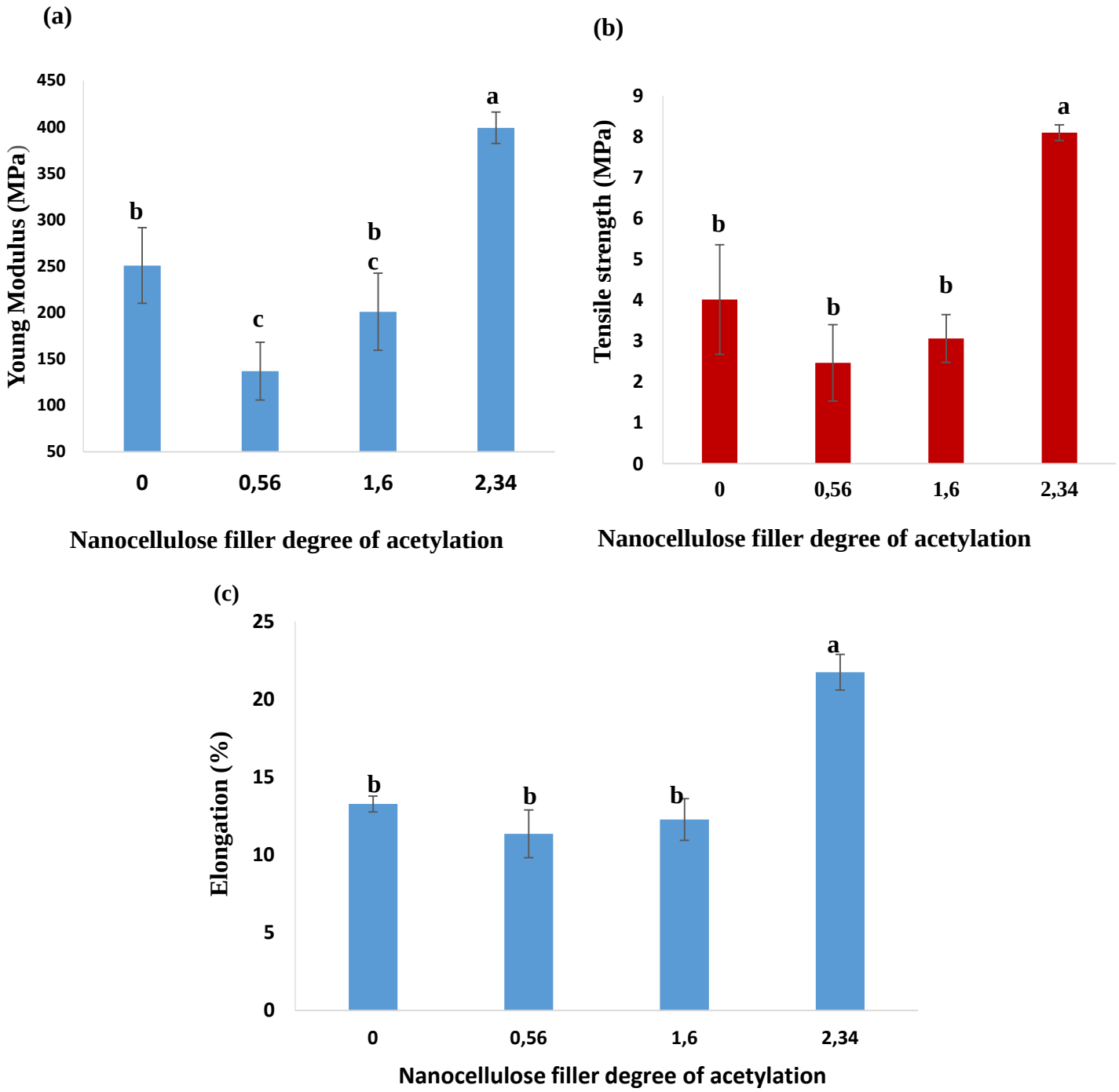


Figure 42: Acetylated hemicellulose-based films (a) Young's modulus, (b) tensile strength and (c) percent elongation as affected by reinforcing with nanocellulose of different degrees of substitution. . The values are means of three measurements. Values with different letters are significantly different ($p < 0.05$). The error bars represent standard deviation

6.11.2. Effect of acetylated nanocellulose loading on reinforcing efficiency of acetylated nanocellulose in acetylated hemicellulose films

The reinforcing efficiency of ACNC in AH (DS 1.7) increased from 0.059 to 6.29 with an increase in ACNC loading from 10 % to 50 % (Figure 43). The increase in reinforcing efficiency with ACNC loading is evidence of the contribution of the ACNC to the stiffness of the hemicellulose matrix (Figure 45) an effect that was reported in previous studies (Osoka and Onukwuli, 2018). Furthermore, the increase in reinforcing efficiency is an indication of the improvement in the AH to ACNC interaction in the biocomposite with the increase in loading % (Figure 43). The reinforcing efficiency of ACNC in AH can be classified as low at 10% fibre loading, medium at 30% ACNC and high for 20%, 40% and 50% ACNC loading based on the classification by Aitomäki and Oksman, (2014).

Considering that the reinforcing efficiency can be a function of either Young's modulus or fibre loading (Aitomäki and Oksman, 2014), the higher reinforcing efficiency with 20% ACNC in the biocomposite than with 10% ACNC loading (Figure 43), is attributed to the relatively higher Young's modulus of the AH composite reinforced with 20% than with 10% ACNC loading (Figure 45). However, the results show that at a loading of 30% ACNC, the Young's modulus was lower than at 20% ACNC loading (Figure 45), which is also reflected in the lower reinforcing efficiency of the biocomposite than the biocomposite with 20% ACNC (Figure 43).

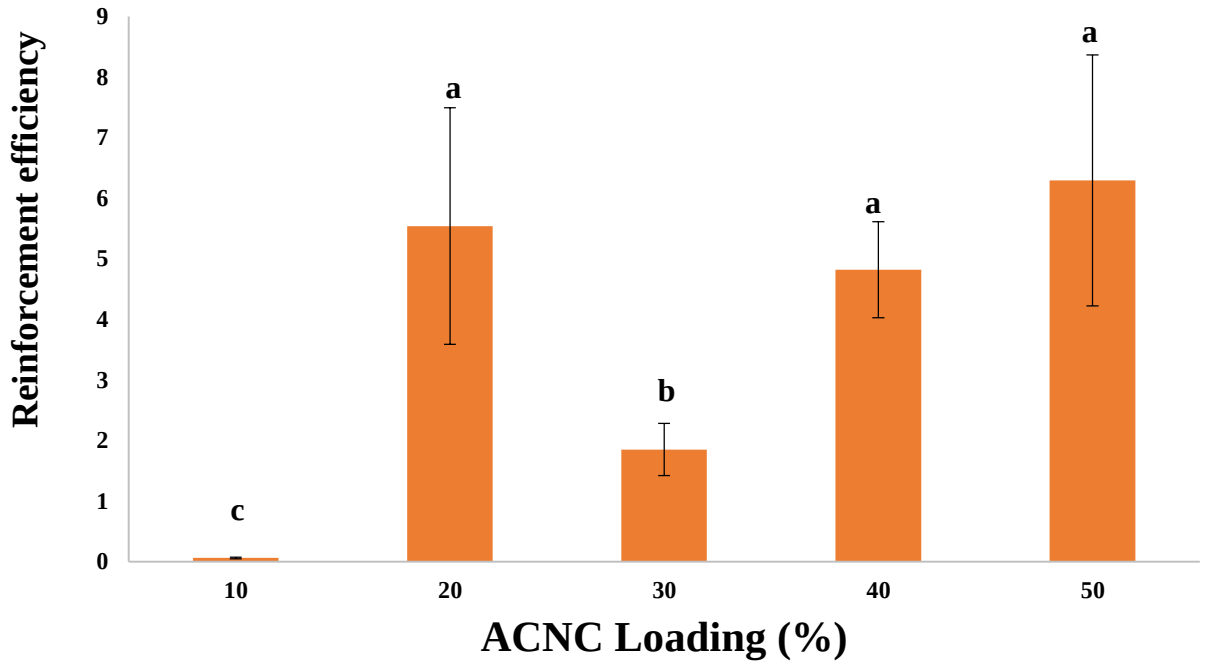


Figure 43: Plot of the reinforcing efficiency of acetylated nanocellulose in acetylated hemicellulose biocomposite at different acetylated nanocellulose loading. ACNC: Acetylated nanocellulose. The values are means of three measurements. Values with different letters are significantly different ($p < 0.05$). The error bars represent standard deviation

An increase in reinforcing efficiency with increase in ACNC loading to 50% suggests that the ACNC was well dispersed in the matrix and that there was excellent bonding between the matrix and reinforcing material (Dong *et al.*, 2017). On the contrary, some researchers have reported a decline in reinforcing efficiency when nanocellulose loading increase to 50% due to an increased interaction of the nanocellulose fibres at higher quantities in composites (Osoka and Onukwuli, 2018). Therefore, ACNC loading of 50% is required to achieve the highest reinforcing efficiency in AH with DS of 1.7.

6.11.3. Effect of acetylated nanocellulose loading on strength efficiency

A positive correlation can be observed for the strength efficiency of AH/ACNC films with an increase in ACNC loading (Figure 44). The increase in the ACNC loading from 10% to 50% contributed to the increase in the tensile strength of the AH/ACNC films as shown by the experimental tensile strength data in Figure 46, hence the increase in strength efficiency with an increase in ACNC loading (Aitomäki and Oksman, 2014). The strength efficiency values of the films with different ACNC loading were all higher than 100% showing that the theoretical model underestimated the tensile strength of the AH/ACNC film. To be specific, there was a positive deviation of the experimentally obtained biocomposite strength from the theoretical predictions by up to 127%, as ACNC loading increased from 10% to 50%. Furthermore, the deviation of the strength efficiency with changes in ACNC loading ranged between 18% and 43%. This therefore, means that the AH/ACNC films has exceptional toughness as their tensile strength surpasses the ideal theoretical model predictions. Similar results have been reported for epoxy reinforced with flax and chitosan reinforced with nanofibrillated cellulose (Aitomäki and Oksman, 2014). The higher experimental tensile strength could be due to other factors not considered in the models such as the reduction in voids in the matrix with increase in filler content (Mehdikhani *et al.*, 2019). Hence increasing the loading of ACNC with a DS of 2.34 effectively improves the tensile efficiency of AH films.

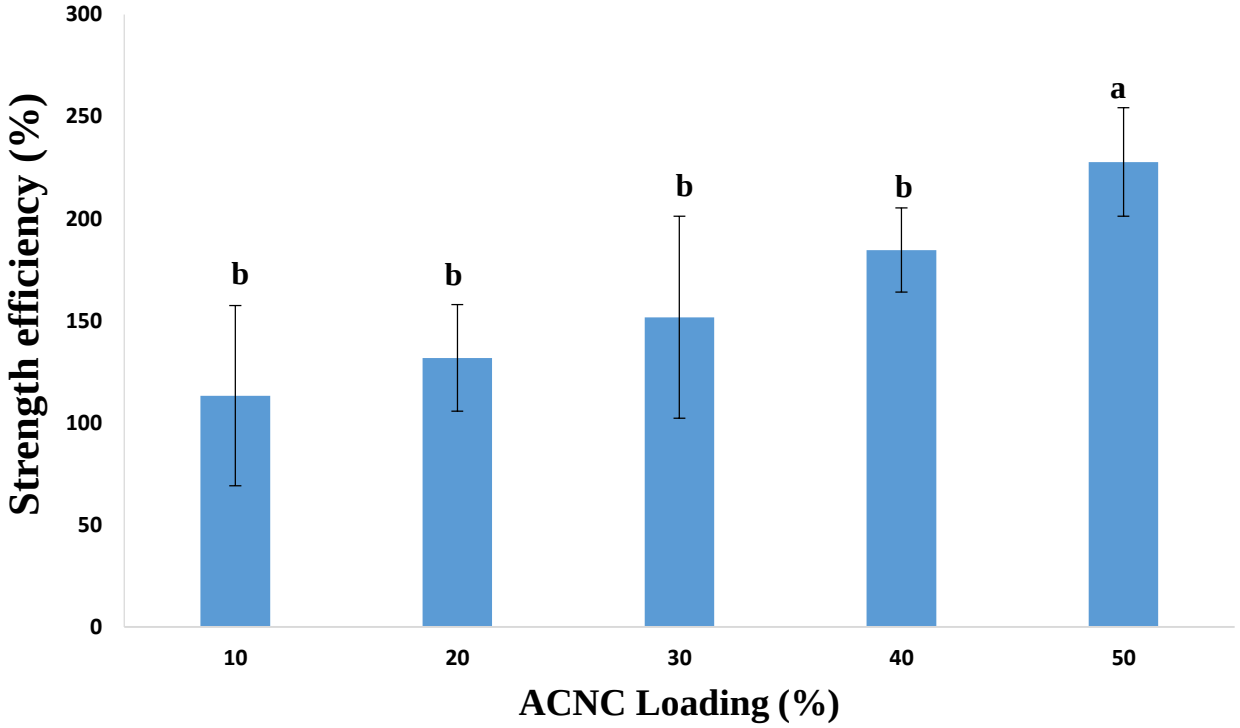


Figure 44:. Plot of the strength efficiency of acetylated hemicellulose/nanocellulose biocomposites at different acetylated nanocellulose loading. ACNC: Acetylated nanocellulose. The values are means of three measurements. Values with different letters are significantly different ($p < 0.05$). The error bars represent standard deviation

6.11.4. Comparison of Young's modulus and tensile strength obtained from experiments to theoretical predictions

The trends in Young's modulus and tensile strength obtained both experimentally and from models are shown in Figure 45 and Figure 46 respectively. It is worth noting that for both tensile strength and Young's modulus the model predicted values and the experimental values increased with an increase in reinforcing nanocellulose loading from 10% to 50%. At ACNC loading less than 10% in AH films, the model predictions of both Young's modulus (Figure 45) and tensile strength (Figure 46) of AH/ACNC films least deviated from the experimental results (deviation less than 100%) when compared to higher ACNC loading (deviation up to 6000 %). This could be due to the uniform dispersion of the short fibres at low ACNC loading, which is one of the assumptions of all the models (Munde and Ingle, 2015). However, an increased deviation of all the experimental results from the model predictions was observed as the reinforcing ACNC loading increased from 10% to 50%. The lack of fit could be due to non-uniform distribution of the fibres in the biocomposite as a result of fibre-fibre interactions and entanglement at increased ACNC loading (Tham *et al.*, 2019). Furthermore considering that all the theoretical models used in this study assume a uniform distribution of reinforcing material in the matrix, the non-uniformity in ACNC distribution in the AH composite resulted in the poor fit of the model to the experimental data

(Chen, 2015; Kundalwal, 2018). Joseph *et al.* (2003) found similar trends when investigating the effect on tensile strength and Young's modulus for polypropylene biocomposites of reinforcing with sisal fibre. Therefore, the theoretical models are best suitable for predicting the mechanical properties of AH/ACNC at ACNC loading lower than 10%.

6.11.4.1. Prediction of Young's modulus

Arranging the models from the best AH/ACNC composite Young's modulus predictor to the least, the following order was observed; Cox-Krenchel> Tsai-Pagano> Halpin-Tsai> isostress> isostrain (Figure 46). The Cox-Krenchel model gave the best Young's modulus predictions for all the ACNC percent loading investigated because of lower percent deviation from experimental values when compared to the other models. All the predicted values from the Cox-Krenchel model had a percent standard deviation of less than 8.5% from the experimental values, whereas, the percent deviation of the other models were Tsai-Pagano 21.5%, Halpin-Tsai 36%, isostress 37%, and isostrain 6000%. Andre *et al.* (2017) also showed that the Cox-Krenchel model was good at predicting the Young's modulus for epoxy/kenaf composites (Andre *et al.*, 2017). The better prediction capabilities of the Cox-Krenchel model when compared to other models has been attributed to it factoring in the dimensions and orientation of the reinforcing fibre in the biocomposite (Kundalwal, 2018). The Tsai-Pagano model was best suited for obtaining the Young's modulus for ACNC loading up to 30% with a percent deviation of less than 10.3%. However, addition of ACNC higher than 30% resulted in the predicted modulus deviating up to 31% below the experimental value (Figure 45). Failure to predict the Young's modulus as shown by the overestimation of the Young's modulus by the Halpin-Tsai model could be due to the model assuming perfect bonding between the AH and ACNC, which ideally, is impossible (Islam and Begum, 2011).

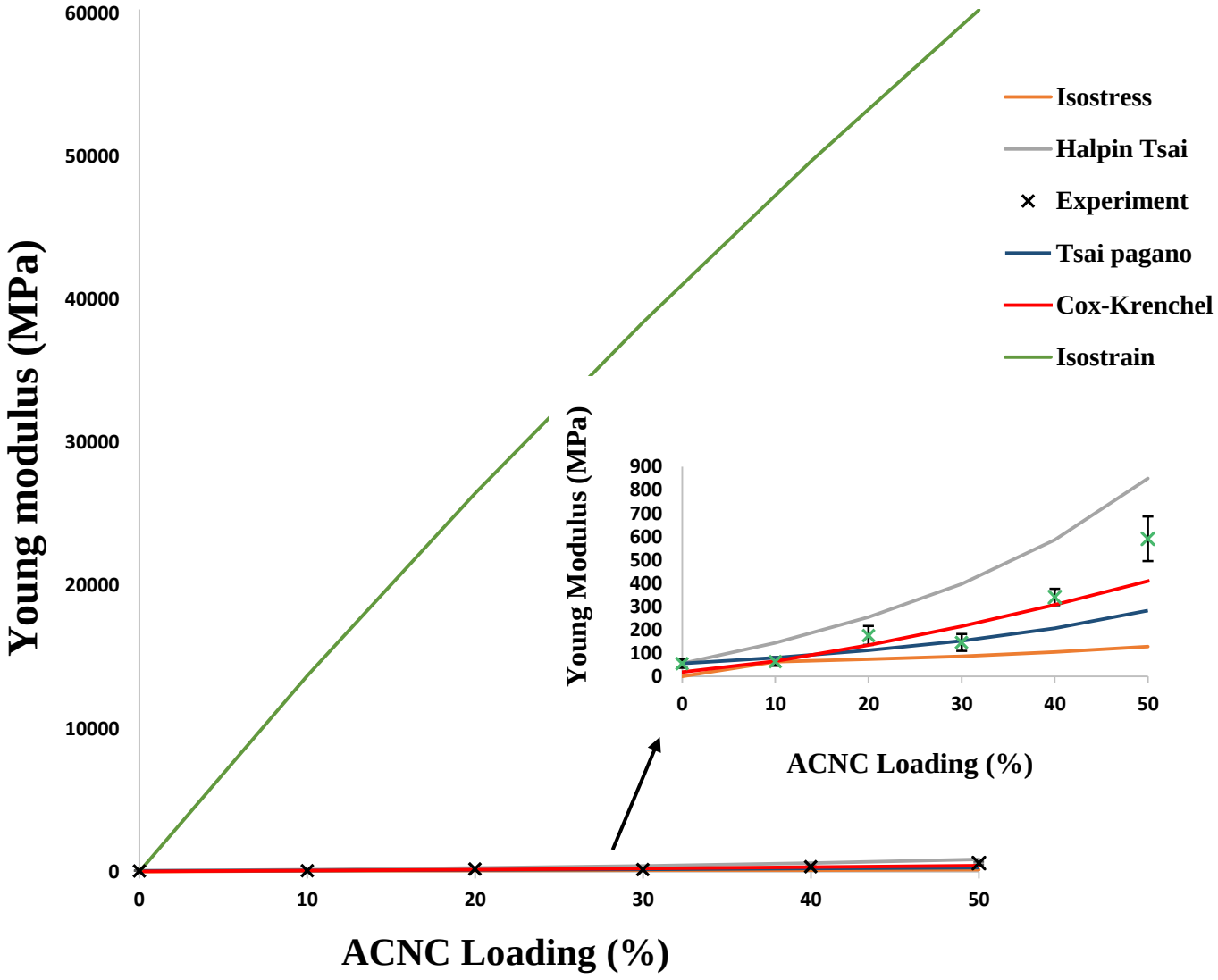


Figure 45: Experimental and theoretical Young's modulus of acetylated hemicellulose/nanocellulose biocomposites at different acetylated nanocellulose loading. ACNC: Acetylated nanocellulose. The error bars represent standard deviation

The isostrain and isostress models present the upper and lower boundaries for Young's modulus that can be obtained from AH/ACNC biocomposites. Isostrain models assume that the reinforcing ACNC are continuous fibres that are arranged longitudinally in the hemicellulose matrix whereas isostress model assumes that the continuous fibres are arranged perpendicular to the matrix (Chen, 2015). The experimental results were closer to the isostress than the isostrain suggesting that the ACNC fibres are mostly arranged perpendicularly to the AH matrix than longitudinally. Therefore, there is potential in the utilisation of the Cox-Krenchel model to predict the Young's modulus of AH/ACNC composites during the design stage.

6.11.4.2. Prediction of the tensile strength

The tensile strength of the biocomposite was best predicted by the Bowyer-Bader model, followed by the Hirsch, ROM, parallel and lastly the series models (Figure 46). The predictions from the Bowyer-Bader model were closer to the experimental results, with the largest percent deviation being 18.2%. The study by Andre *et al.* (2017) reported that the Bowyer-Bader model was the best fitting after recording a percentage deviation from the actual results of less than 18.6% (Andre *et al.*, 2017). This model has been known to give more accurate predictions than the other tensile strength models because it takes into account two of the most important factors that affect mechanical properties, namely fibre length and orientation in the matrix (Raharjo *et al.*, 2018). The parallel model (43 524%) had the highest deviation from the experimental results followed by the series model with 55.4%, ROM with 50.3% then the Bowyer-Bader model with 18.2%. Comparing the series and parallel models to the experimental results confirms that the orientation of the ACNC in the AH was perpendicular to the AH film as the experimental results are closer to the parallel model than the series model. Therefore, the Bowyer-Bader model is the most suitable among the studied models for tensile strength prediction of AH reinforced with ACNC.

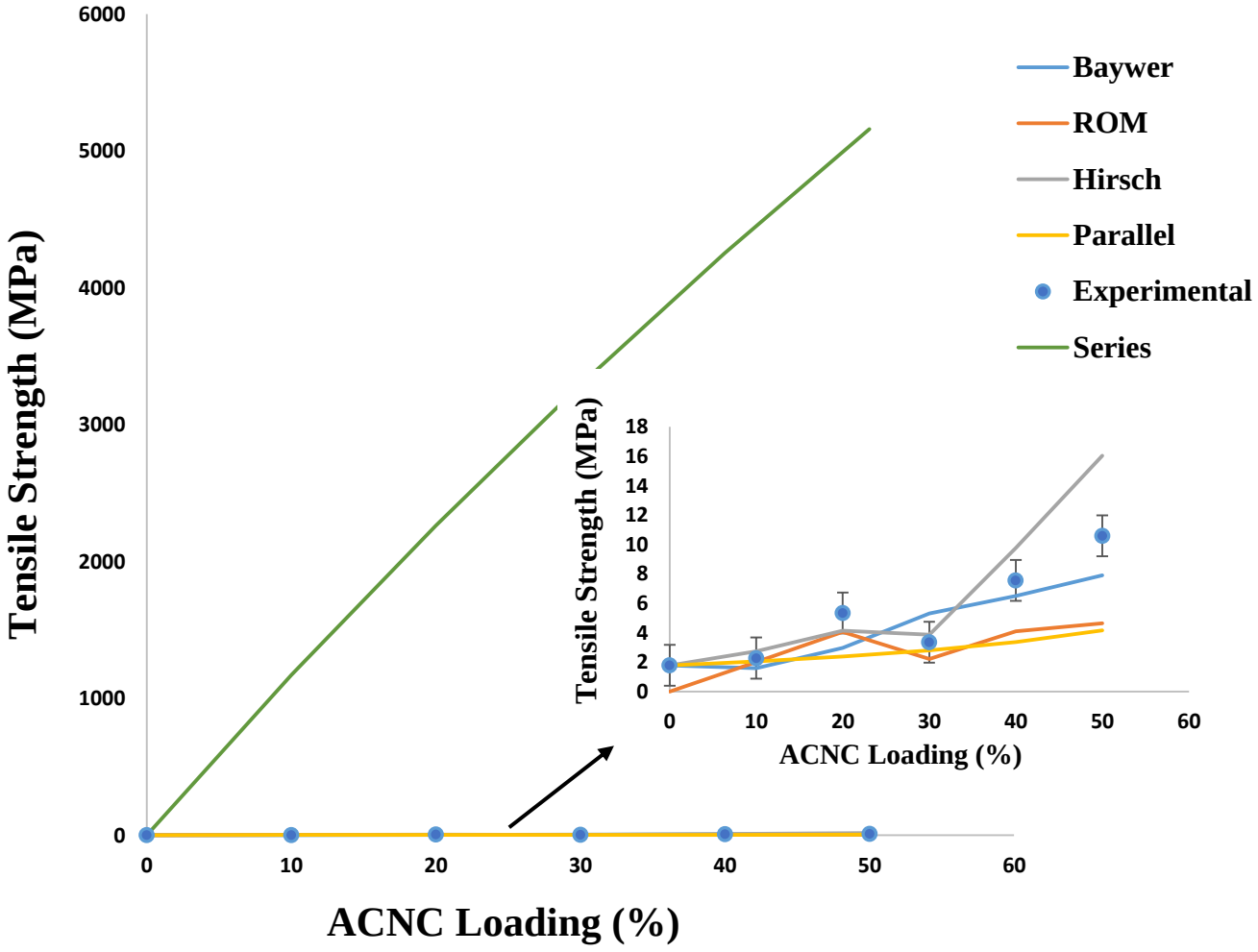


Figure 46: Experimental and theoretical tensile strength of acetylated hemicellulose/nanocellulose biocomposites at different acetylated nanocellulose loading. ACNC: Acetylated nanocellulose. The error bars represent standard deviation

6.12. Conclusions

Increasing the DS of ACNC significantly improves the mechanical properties of reinforced AH films. ACNC exhibits a high reinforcing efficiency and strength efficiency with increase in ACNC loading in AH based films. The Tsai-Pagano model and the Bowyer-Bader model were the most suitable models for predicting Young's modulus and tensile strength of AH/ACNC biocomposites respectively. Both the experimental values and theoretical model predictions showed a positive correlation of AH/ACNC biocomposite tensile strength and Young's modulus with increasing in reinforcing ACNC loading. This study contributes towards finding the best matrix for utilising nanocellulose as a reinforcement material. Future work focuses on the application of the AH/ACNC films as a food packaging material.

6.13. Paper Title: "Enhancing the functional properties of acetylated hemicellulose films for active food packaging using acetylated nanocellulose reinforcement and polycaprolactone coating"

Published in the journal of Food packaging and shelf life (impact factor 3.67)

6.14. Abstract

AH/ACNC films coated with PCL films, were evaluated as active packaging for aqueous, alcoholic, fatty and acidic food. The effects of nanocellulose loading (0 - 50%), degree of acetylation (DS) (0-2.34) and PCL coating (0.3 g/mL) on hydrophobicity and solubility of AH films in food simulants, were investigated. In addition, AH/CNC/PCL films were doped with polyphenols and their antioxidant release (temperature 5 ^{o}C - 40 ^{o}C, time - 48 h) into food simulants was evaluated experimentally and by modelling (Migratest software). Increasing ACNC DS and loading, combined with a PCL coating increased films' hydrophobicity (24.59^{o} to 82.48^{o}) and reduced film solubility in all the simulants (~82.8%). The release of polyphenols by the films was highest and best predicted using Migratest software for the fatty food simulant. Therefore, these films can be used as active packaging for fatty foods. Furthermore, Migratest modelling can be used to predict film performance during film design.

Keywords: Acetylated hemicellulose; Acetylated nanocellulose; Acetylation; Active packaging; Mango polyphenols; Controlled release

6.15. Introduction

In recent years, several studies have focused on finding alternative raw materials for developing food packaging films to replace the non-degradable petroleum-based packaging material. Hemicellulose has gained attention as a possible substitute for synthetic packaging material such as LDPE (Peng, Peng, Xu and Sun, 2012; Li and Pan, 2018). However, the utilisation of hemicellulose-based films as food packaging material is limited to dry foods due to the high solubility of hemicellulose in wet food media. Modification processes such as benzylation, fluorination and acetylation have been attempted to enhance the hydrophobicity of hemicellulose, with the aim of widening the application of hemicellulose films (Chen *et al*., 2014; Farhat *et al*., 2017; Xu *et al*., 2008). Among the aforementioned strategies applied to minimise the solubility of hemicellulose-based films in food media, acetylation is considered the safest and most efficient method for modifying polymers for food packaging material development (Egüés *et al.*, 2014).

In order to further improve the hydrophobicity of AH films, ACNC is added as a reinforcing material (Gordobil *et al.*, 2014). Research on the development of AH films reinforced with ACNC is still limited to the blending of AH with ACNC that has a DS of 0 and 0.54 (Gordobil *et al.*, 2014). However, there is potential in further enhancing the hydrophobicity of AH films by reinforcing them with ACNC that has a DS above 0.5. Increasing the DS of ACNC reduces the number of hydroxyl groups in the film, available for hydrogen bonding with water, while increasing hydrophobic acetyl groups (Ayoub *et al.*, 2013; Akkus *et al.*, 2018). The hydrophobic groups act as a barrier to the penetration of moisture through the film (Khodaeimehr *et al.*, 2018), hence, inhibiting the accumulation of water in the film (Ayoub *et al.*, 2013). In addition, reinforcing the AH films with higher quantities of hydrophobic fillers, can reduce the film surface area available to bond with water molecules (Siracusa *et al.*, 2018). Thus, increasing the DS and loading of ACNC in AH films, can be a strategy in developing films with enhanced water barrier properties. The ACNC subsequently enhances the mechanical properties of the AH films (Gordobil *et al.*, 2014).

AH/ACNC films have been recommended for food packaging, based on the barrier properties (oxygen barrier, gas barrier) and mechanical properties (Young's modulus and tensile strength) without testing their stability when in contact with food media (Farhat *et al.*, 2016). Solubility testing of packaging material in aqueous, acidic, alcoholic and fatty food simulants is among the recognised approaches to evaluate the stability of packaging material when in contact with different food media (EU, 2011; Silva, Cruz, García, Franz, & Losada, 2007). This test procedure has been done on both biopolymers and synthetic-based films. Examples of the films include: pectin-carboxyl methylcellulose films, starch triacetate films, LDPE and Ethylene vinyl alcohol (Fang & Vitrac, 2017; Ibarra, Sendón, García-Fonte, Losada, & Quirós, 2019; Traistaru, Rivis, Moldovan, &

Menelaou, 2013; Yu, Hu, & Wang, 2017; Zhu, Li, Huang, Chen, & Li, 2014). Biobased films are water soluble, hence they disintegrate when exposed to food containing water. The dissolution of the film in food can affect the integrity of the film and the quality of the packaged food (Bhunia *et al.*, 2013). Furthermore, the dissolved packaging material components can be a health hazard to consumers when in concentrations above the allowable limit (Gavriil *et al.*, 2018). Thus, assessment of the solubility of the AH/ACNC film in food is necessary.

Recent developments in food packaging materials include the production of films capable of releasing active compounds into packaged food (active packaging) to increase food shelf life (Muriel-Galet *et al.*, 2015; Cesur *et al.*, 2017). The active compounds eliminate oxidising agents (for example free radicals) and microorganisms (for example bacteria) responsible for food spoilage, thereby prolonging food shelf life (Ebrahimi *et al.*, 2019). Natural antioxidants such as tea polyphenols, tocopherol, and anthocyanins have been incorporated into both synthetic (LDPE) and biobased polymers (starch, pectin), and the release of these antioxidants into food was experimentally and theoretically assessed (López De Dicastillo, Bustos, Guarda, & Galotto, 2016; Pereira, de Arruda, & Stefani, 2015). Theoretical evaluation of antioxidant release by packaging material is often done using software packages such as AKTS SML and Migratest EXP, which were developed based on Fick's second law of diffusion. The predictions made by the aforementioned software programs are compared to experimental data to assess whether these models can be used for packaging material design (Duan, Chen, Zhu, Zhang, & Zhang, 2013; Poças, Oliveira, Oliveira, & Hogg, 2008).

More importantly, it is crucial that active packaging materials have controlled release of antioxidants into food with time, so as to minimise the accumulation of antioxidants in food at the same time facilitating the removal of food spoilage agents as they are generated (Uzunlu & Niranjan, 2017). Antioxidant concentration beyond the allowable limit (60 mg/kg of food) in food can be a health hazard to consumers (EU, 2011). A coating can be applied to food packaging material to control the migration of antioxidants into food, thereby ensuring a slow release of antioxidants from packaging materials (Yu *et al.*, 2017). Polymer coatings such as PCL, have been applied to packaging to prevent the direct contact of the packaging material with food as well as to increase the distance which the entrapped active compounds have to travel to reach the surface of the food, thus, facilitating a slow release of the entrapped bioactive agents into food (Ghavimi, Ebrahimzadeh, Solati-Hashjin, & Abu Osman, 2015). In addition, coatings having been reported to minimise film solubility and improve both the surface properties and mechanical properties of packaging material (Maciel *et al.*, 2014; Rešček *et al.*, 2018). Therefore, there is potential in

developing AH/ACNC based active packaging with controlled release capabilities, and enhanced mechanical and barrier properties, by coating the films with PCL.

The controlled release of chemicals from hemicellulose-based material has only been tested with dry food. Previous research has investigated the utilisation of a hemicellulose coating to prevent the papermaking residual chemicals in recycled paper from migrating into tenax, a dry food simulant. Depending on the surface charge of the chemicals, the hemicellulose chelated some of the chemicals, thus, restricting their migration into the food (Grondal, 2013). However, the ability of hemicellulose-based films, particularly the AH films, to encapsulate and release antioxidants into wet food, has not yet been evaluated. Therefore, in order to diversify the application of the hemicellulose-based films, it is necessary to investigate the antioxidant release by the hemicellulose-based films in different wet food environments (acidic, aqueous, alcoholic and fatty food), to ascertain the film behaviour in these environments.

The overall objective of the study was to develop AH-based active packaging films and test their application as a packaging material for wet foods. The effects of increasing ACNC DS beyond 0.54, ACNC loading (10% - 50%) and applying a PCL coating on the AH/ACNC film's hydrophobicity (WCA), mechanical properties (Young's modulus, tensile strength and percent elongation), and solubility in food simulants (aqueous, acidic, alcoholic and fatty), were investigated. LDPE films were used as a benchmark. In addition, the ability of AH-based films to entrap and release bioactive compounds (mango peel polyphenols) into food simulants, was investigated both experimentally and by modelling, using Migratest Exp software.

6.16. Materials and methods

6.16.1. Chemicals

NaOH, ethanol, sulphuric acid, HCl, phenolphthalein indicator and sodium carbonate were sourced from Kimix Chemical & Lab Supplies (Cape Town, South Africa), and carbazole, glucuronic acid, acetic anhydride, acetic acid, DMSO, sugar standards (arabinose and xylose), gallic acid, Folin Ciocalteu reagent, dichloromethane, PCL (molecular weight 80 000), and DPPH were purchased from Sigma Aldrich (Germany). University of Maine, Orono, U.S.A supplied the freeze-dried nanocellulose nanocrystals. LDPE crystals were sourced from Sasol (South Africa). All the chemicals were of analytical grade. Wheat straw was obtained from wheat straw farmers in Cape Town, South Africa.

6.16.2. Sample preparation

6.16.2.1. Hemicellulose extraction and acetylation

Hemicellulose was extracted from wheat straw according to the method in section 5.4.4. The recovered hemicellulose had a UAC of 8.91%, lignin content of 4.6% and an arabinose/xylose ratio of 0.087. Hemicellulose modification by acetylation was carried out using the method in section 5.4.5

6.16.2.2. Nanocellulose acetylation

Nanocellulose was acetylated to different DS according to the method in section 5.4.6.

6.16.2.3. Preparation of hemicellulose films

The hemicellulose-based films were prepared according to a method highlighted by Egüés *et al*., (2014). AH/ACNC films reinforced with ACNC of different DS were prepared by dissolving 375 mg of AH and 125 mg ACNC in 20 mL of DMSO by heating for 6 h at 50 ^{o}C in a shaking water bath. AH/ACNC films with different ACNC loading were formulated by dissolving concentrations of ACNC ranging from 10% to 50% (based on the total film solids weight, 500 mg), together with AH in 20 mL of DMSO. The mixture was heated in a water bath at 50 ^{o}C for 6 h. The hemicellulose-based films were then created by solvent casting the homogenous mixture in 80 mm glass Petri dishes before oven-drying at 50 ^{o}C for two days (Egüés *et al.*, 2014). A coating of PCL on the AH/ACNC films and AH/ACNC/Polyphenol films was formed by dipping films in 100 mL of 0.03 g/mL PCL/dichloromethane solution for 5 s. No polyphenols were added to the PCL coating. The coated films were then oven-dried at 25 ^{o}C for 24 h. Film thickness was measured using an Elcometer (model 456CFSS). The thickness of the films was controlled by maintaining the quantity of film mixture and size of Petri dish used for solvent casting.

6.16.2.4. Preparation of low density polyethylene films

LDPE was first milled to a powder prior to making films using a laboratory retch mill (Retch ZM200 mill with a 2 mm circular blade). LDPE films were developed by injection moulding (Thermo scientific Haake mini Jet II from Lasec, South Africa). The injection and mould temperature were set at 130 ^{o}C and 100 ^{o}C respectively. The moulding was carried out at 450 bars for 20 minutes.

6.16.3. Analytical methods for determining the physicochemical properties of hemicellulose, nanocellulose and hemicellulose-based films.

6.16.3.1. Determination of the degree of acetylation of hemicellulose and nanocellulose

The DS of AH and ACNC was determined based on the method described in section 5.4.8.

6.16.3.2. Determination of water contact angle of acetylated hemicellulose-based films

The WCA of the AH-based films was determined using the method in section 5.4.8

6.16.4. Determination of the functional properties of acetylated hemicellulose-based films

6.16.4.1. Assessment of film solubility in the food simulants

The solubility of AH/ACNC films in the aqueous (deionised water), acidic (3% acetic acid), alcoholic (10% ethanol) and fatty (95% ethanol) food simulants, was determined according to a method stated by López De Dicastillo *et al*. (2011). The AH/ACNC films, cut into strips with a length and width of 2 cm x 2 cm, were totally immersed in food simulants (30 mL) for 24 h at 25 ^{o}C . After that the strips were oven dried at 40 ^{o}C for 24 h. The solubility of the film was calculated as the percentage weight of the film that dissolved in the simulant after 24 h.

6.16.4.2. Determination of antioxidant release by the acetylated hemicellulose-based films and low density polyethylene films

A mango peel polyphenol extract, with total polyphenol content of 82.04 mg/g GAE and antioxidant activity of 93.4%, was used as the antioxidant source. AH/ACNC/Polyphenol/PCL were developed by immersing the AH/ACNC film in the polyphenol extract for 1 min. The films were then allowed to dry in an oven for 1 day at 25 ^{o}C. The films were able to absorb 2.89 mg/g GAE of the mango polyphenols (0.057 mg/cm^2). A PCL coating was applied to the films using the procedure described in section 6.16.2.3 Approximately, 3 mL of the polyphenol extract was added to the LDPE powder prior to forming the films using the method in section 6.16.2.4. The LDPE films had a polyphenol content of 2.83 mg/g GAE.

The film polyphenol release into the food simulants was determined according to a method formulated by Yu *et al*., (2017). Strips of the AH/ACNC/Polyphenol/PCL films and LDPE/polyphenol films, with length and width of 2 cm, were immersed in 30 mL of food simulant and incubated at 5 ^{o}C, 25 ^{o}C and 40 ^{o}C for 48 h. A sample of the simulant was taken periodically to analyse polyphenol content and antioxidant activity based on the method highlighted in section 4.4.3.

6.16.5. Modelling the release of mango polyphenols by the acetylated hemicellulose-based films into the food simulants

The migration of mango peel polyphenols from the AH/ACNC/PCL/Polyphenol films into food simulants was modelled using the Migratest EXP software. The diffusion coefficients for the polyphenols in the hemicellulose-based films as affected by the simulants were calculated using Equation 46 highlighted in section 6.5.2. The partition coefficients of the films were calculated based on Equation 47 which is in section 6.5.2.

The polyphenol release by the AH/ACNC/Polyphenol films was modelled for the temperatures of 5 ^{o}C, 25 ^{o}C and 40 ^{o}C, and time ranging from 0 h to 48 h. Migration modelling was carried out for the aqueous, acidic, alcoholic and fatty simulants. The simulant volume was specified as 30 mL. The AH/ACNC/Polyphenol/PCL film length, width and thickness were 2 cm x 2 cm x 0.0358 cm. The films were specified as having three layers, namely, the AH/ACNC layer with a thickness of 0.250 mm and a PCL layer with a thickness of 0.054 mm on either side of the AH/ACNC layer. PCL density was stated as 1.45 g/cm^3 and the density of AH/ACNC films, 0.785 g/cm^3, was experimentally calculated from the densities of AH and ACNC. The mango polyphenols molecular weight (300.07) was calculated, based on method reported by Shivapuji, (2019), considering the molecular weights and composition of the individual polyphenol type present in the mango peel polyphenol extract.

6.16.6. Imaging of hemicellulose-based films using field emission scanning electron microscopy

Images of the hemicellulose-based films were captured using a Carl Zeiss Merlin FESEM equipped with a GEMINI II column. The films were first coated with gold and secured on a stub using double sided tape, prior to imaging. Then an in-lens detector set at working distance = 4.5 mm, probe = 100 Pa and EHT = 3.00 kV, to capture the surface profiles of the films. In order to obtain images to analyse the dispersibility of ACNC in AH films the in-lens detector was set at a working distance = 4.5 mm, probe = 100 Pa and EHT = 3.00 kV. Imagej software was used to analyse the SEM images to determine the dispersibility of ACNC in AH films.

6.16.7. Structural characterisation of hemicellulose-based films using Fourier transform infrared spectroscopy (FTIR)

The structural characteristics of the hemicellulose based films was analysed using the method highlighted in section 6.5.3.1.

6.16.8. Statistical analysis

All analyses were carried out in triplicate. Comparison of the means obtained was performed using the Duncan multiple stage analysis in Statistica version 7, 13.2 software.

6.17. Results and discussions

6.17.1. Effect of nanocellulose degree of substitution on acetylated hemicellulose/nanocellulose film thickness

Acetylation of nanocellulose resulted in ACNC with DS ranging from 0 to 2.34 and the means of the DS were significantly different ($p < 0.05$) from each other (Table 20). The nanocellulose DS had an effect on the thicknesses of the AH/ACNC films, that is, the film thickness decreased from

81.47 to 53 µm upon increasing the DS of the reinforcing ACNC from 0 to 2.34 (Table 20). This could be due to the DS of the ACNC affecting the dispersion of ACNC in the film matrix, as evidenced by a decline in distance between the ACNC particles in the film from 447.71 nm to 6.35 nm when ACNC DS decreased from 2.34 to 0 (Table C1, Figure C1). At a lower DS, the ACNC particles entangled and agglomerated, compromising the dispersion of the ACNC in the AH matrix (Table C1) (Dong, Yan, Jin, & Li, 2017; Fasihnia, Peighambardoust, & Peighambardoust, 2017), thereby increasing film thickness and reducing the uniformity of the films (Table C1).

Table 20: Effects of acetylated nanocellulose degree of substitution on hemicellulose based-film thickness

Film	ACNC DS	Film thickness (µm)	Standard deviation
AH/ACNC(0)	0[a]	81.47	8
AH/ACNC(1)	0.55[b]	70.74	7.82
AH/ACNC(2)	1.6[c]	65.50	4.13
AH/ACNC(3)	2.34[d]	53	2.51

Film thickness was measured randomly measured on the film surface using an Elcometer (model 456CFSS).

AH: Acetylated hemicellulose, ACNC: Acetylated nanocellulose, DS: Acetylation degree of substitution. The values are means of three measurements. Values with different letters are significantly different ($p < 0.05$).

6.17.2. Effect of nanocellulose degree of substitution on acetylated hemicellulose film hydrophobicity and solubility in food simulants

Reinforcing AH (DS 1.7) with ACNC of higher DS (increasing DS from 0 to 2.34), reduced the solubility of the hemicellulose-based films, from 30.88% to 16.30%, in the food simulants (Figure 47a). The observed trend can be attributed to the reduction in the hydroxyl group content of the films when the ACNC DS increased. Replacing the hydroxyl groups in the nanocellulose with hydrophobic acyl groups, limits hydrogen bonding of the films with water, thereby reducing the solubility of the film in food simulants (Fang, Sun, Tomkinson, & Fowler, 2000). Furthermore, the acetyl groups of the ACNC form strong hydrophobic networks with acetyl groups in AH which restrict water absorption and film swelling when in water-based simulants, thus, reducing the solubility of the film into the simulant (Fortunati *et al.*, 2012). Films reinforced with ACNC that has a low DS are likely to form hydrogen bonds with water in simulants because of the high hydroxyl groups content, thus, promoting the dissolution of the films in simulants (López De Dicastillo *et al.*, 2011). The films were generally least soluble in the fatty food simulant when compared to the other simulants because the fatty food simulant has a lower water content (5%) than the other simulants (90-100%) (Figure 47). Hence, the fatty food simulant has a lower concentration of hydroxyl groups available for bonding with the films than the other simulants (Fortunati *et al.*, 2012). Therefore, AH

films reinforced with ACNC have potential application as packaging material for fatty foods when compared to the aqueous, alcoholic and acidic food.

A significant reduction ($p < 0.05$), of up to 38.16%, in solubility of AH based films in all the food simulants was observed when the DS of the reinforcing ACNC was increased from 1.6 to 2.34 (Figure 47a). The results suggest that the compatibility of the AH matrix and the ACNC filler was boosted by the DS of nanocellulose (Fortunati *et al.*, 2012; Dong *et al.*, 2017). On the other hand, there was no significant reduction ($p > 0.05$) in the solubility of AH/ACNC films when the DS of ACNC increased from 0 to 1.6 (Figure 47a). The phenomenon suggests that there is a minimum DS in nanocellulose which inhibits the solubility of AH/ACNC films in the simulants. Most likely, for such low DS, the hydrophobic networks present in the films were not sufficient to prevent the films from forming hydrogen bonding with water in the simulants. Nevertheless, the AH/ACNC films reinforced with ACNC of DS ranging from 0 to 1.6, exhibited functional properties suitable for packaging fatty foods as evidenced by the least solubility of these films into the fatty simulant when compared to the aqueous, alcoholic and acidic food simulants (Figure 47a).

The hydrophobicity of the AH/ACNC films was enhanced by increasing the DS of the ACNC reinforcement as evidenced by the increase in WCA of the films from 24.59^{o} to 62.68^{o} as the nanocellulose DS increased from 0 to 2.34 (Figure 47b). The AH films containing ACNC with DS 2.34 had a WCA significantly higher ($p < 0.05$) than that of AH films reinforced with ACNC which had DS ranging from 0 to 1.6.

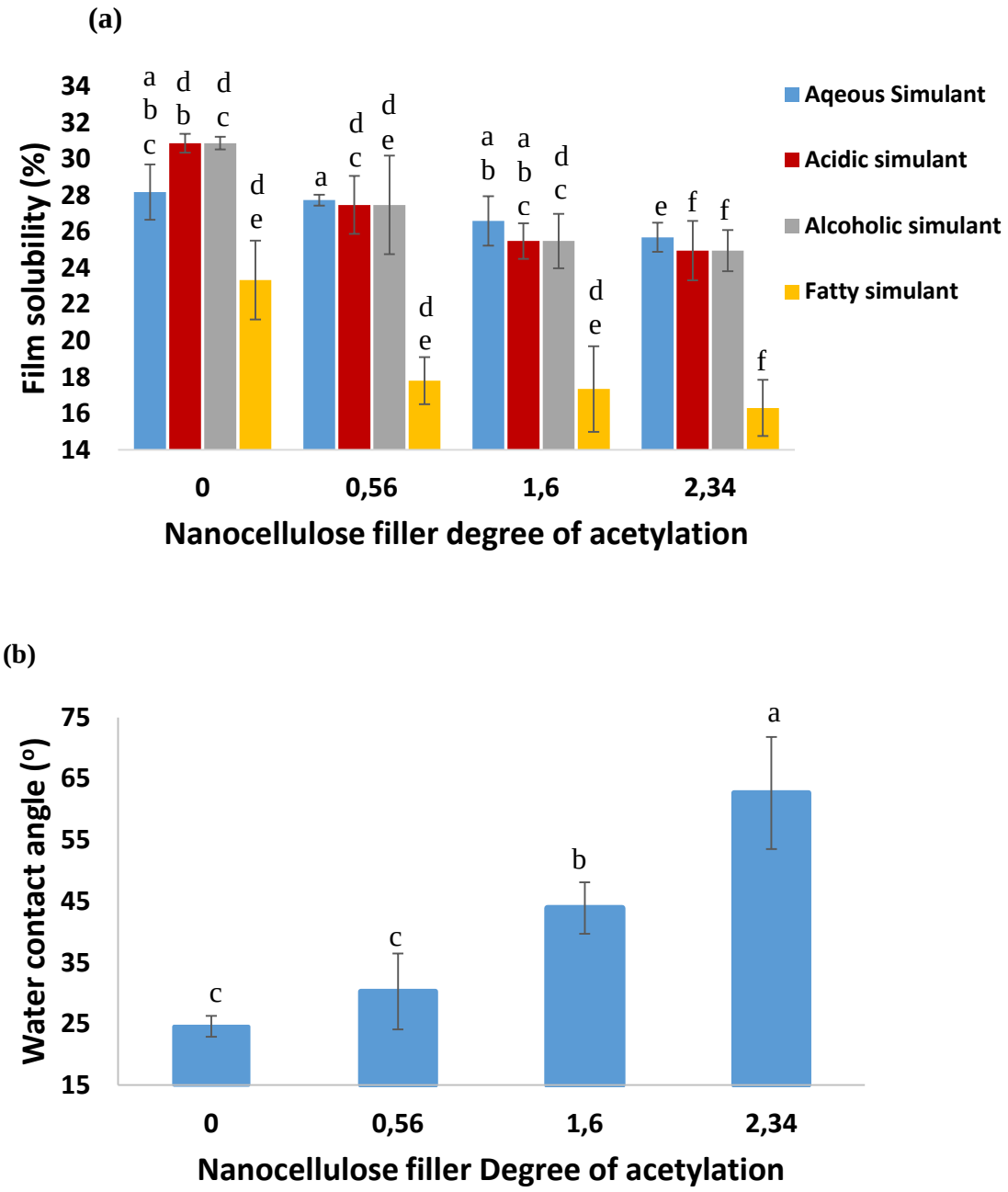


Figure 47: Hemicellulose-based film (a) solubility in food simulants and (b) water contact angle as affected by varying the degree of acetylation of the reinforcing nanocellulose. The values are means of three measurements. Values with different letters are significantly different ($p < 0.05$). The error bars represent standard deviation

Other researchers have also reported that the hydrophobicity of hemicellulose films can be enhanced by incorporating hydrophobic fillers (Fortunati *et al.*, 2012; Gordobil *et al.*, 2014). However, the WCA of the films reinforced with ACNC of DS ranging from 0 to 1.6 were statistically the same ($p > 0.05$), suggesting that this acetylation DS range was not sufficient to cause a significant increase in the hydrophobicity of the films. Therefore, the phenomenon of increasing the hydrophobicity of AH-based films by the addition of ACNC would only apply when the minimum DS has been achieved, which in this case should be greater than 1.6. In addition, other factors such as dispersion, loading, and compatibility of the reinforcing material and the matrix play a role in the overall hydrophobicity of the biocomposites (Mehdikhani *et al.*, 2019).

6.17.3. Effect of nanocellulose loading on acetylated hemicellulose film hydrophobicity and solubility in food simulants

Increasing the ACNC (DS 2.34) loading from 10% to 50% in AH films (DS 1.7) led to a reduction in solubility of the films in the food simulants, from 24.12% to 12.57% (Figure 48a). The observed results can be attributed to an increase in the formation of hydrophobic bonds between hemicellulose and nanocellulose, as nanocellulose loading increased, leading to the creation of hemicellulose-nanocellulose networks which shielded the hydroxyl groups in the film from interacting with the simulant (Huang *et al.*, 2018). In addition, regardless of the ACNC loading, the AH films were least soluble (as low as 12.57%) in the fatty food simulant when compared to the other simulants (Figure 48a).

An improvement in hydrophobicity of the AH/ACNC films, depicted by an increase in WCA from 18.97 to 68.29^{o}, was realised when the ACNC loading increased from 10% to 50% (Figure 48b). The increase in hydrophobicity of the films can be attributed to the reduction in film surface area available for hydrogen bonding with water as the quantity of ACNC in the hemicellulose matrix increased. Peighambardoust, Peighambardoust, Pournasir, & Pakdel, (2019) highlighted that the inclusion of a more hydrophobic filler in a film matrix enhances the hydrophobicity of the film. Similar results were reported by Pereira *et al*. (2014), when they observed that increasing nanocellulose loading in polyvinyl alcohol composites improved the WCA. Therefore, ACNC loading is an important factor that can be manipulated when aiming at developing AH/ACNC films with improved moisture barrier properties.

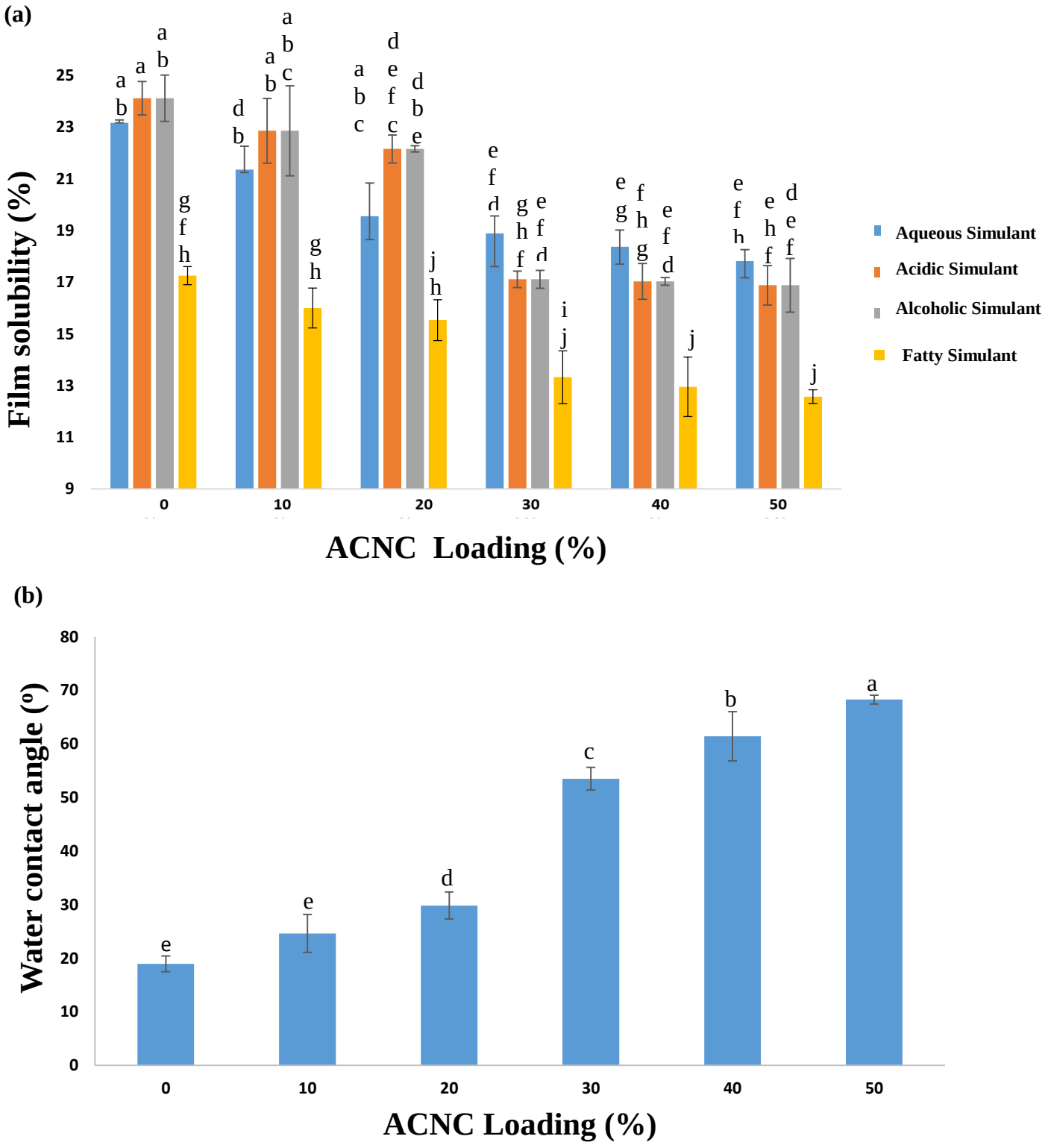


Figure 48: Hemicellulose-based films (a) solubility in food simulants, (b) water contact angle as affected by varying the reinforcing acetylated nanocellulose loading. The values are the means of three measurements. Values with different letters are significantly different ($p < 0.05$). The error bars represent standard deviation. The ACNC loading is the weight percent of nanocellulose in the film.

6.17.4. Effect of polycaprolactone coating, acetylated nanocellulose and polyphenols on the mechanical and functional properties of acetylated hemicellulose-based films

The tensile strength of AH/ACNC based films was similar to that of LDPE films ($p > 0.05$) (Table 21). However, the Young's modulus of the AH/ACNC films was higher than that of LDPE by 58.6% (Table 21). On the other hand, the percent elongation of the AH/ACNC (15.49%) (Table 21) and WCA (68.29^{o}) could not match those of the LDPE (percent elongation of 1621.22% and WCA of 84.91^{o}) (Table 21). Thus, reinforcing AH with ACNC alone is not enough to develop AH-based films that have all functional properties similar to LDPE films (Table 21). A PCL coating further improved the hydrophobicity of the AH/ACNC films from 68.29^{o} to 82.48^{o} (Figure 49). The PCL is inherently hydrophobic (82.91^{o}), thus, its use as a coating improved the water barrier properties of the AH/ACNC films (Zhou *et al.*, 2013). However, there was no significant difference ($p < 0.05$) in the tensile strength and elongation between the PCL coated AH/ACNC films and the uncoated AH/ACNC films (Table 21). The lower Young's modulus of PCL (280.30 MPa) when compared to AH/ACNC films (590.15 MPa) led to a significant reduction ($p < 0.05$) in the Young's modulus of the coated AH/ACNC films (335.33 MPa) (Table 21). Consequently, the coating resulted in a AH/ACNC film with a Young's modulus closer to that of the LDPE films (244.46 MPa).

Table 21: Comparison of mechanical properties and hydrophobicity of acetylated hemicellulose-based films with polycaprolactone films and low density polyethylene films

Film type	Young's Modulus (MPa)	Tensile strength (MPa)	Elongation (%)	Water contact angle (^{o})	Film thickness (mm)
PCL	$280.30^{bc} \pm 52.28$	$13.61^{a} \pm 2.25$	$43.90^{a} \pm 1.76$	$82.91^{a} \pm 0.46$	0.560 ± 0.031
AH/ACNC	$590.15^{a} \pm 95.86$	$10.59^{ab} \pm 1.24$	$15.49^{a} \pm 3.19$	$68.29^{b} \pm 0.81$	0.250 ± 0.045
AH/ACNC/PCL	$335.33^{bc} \pm 21.14$	$6.72^{b} \pm 0.65$	$16.01^{a} \pm 2.01$	$82.48^{a} \pm 6.73$	0.4345 ± 0.082
AH/ACNC/Polyphenols/PCL	$409.91^{c} \pm 14.46$	$8.47^{ab} \pm 1.44$	$15.75^{a} \pm 0.13$	$63.78^{b} \pm 4.48$	0.3585 ± 0.024
LDPE 1	$244.46^{b} \pm 63.51$	$11.07^{ab} \pm 3.97$	$1621.22^{b} \pm 597.02$	$84.91^{a} \pm 2.28$	0.044 ± 0.0035
LDPE 2	$220.41^{b} \pm 46.56$	$14.98^{a} \pm 1.70$	$200.02^{c} \pm 46.72$	$86.1^{a} \pm 0.75$	0.486 ± 0.011

The values are the means of three measurements. Values with different letters are significantly different ($p < 0.05$) from each other. LDPE 1 is a commercial LDPE film and LDPE 2 was experimentally developed. PCL: Polycaprolactone, AH: Acetylated hemicellulose, ACNC: Acetylated nanocellulose, LDPE: Low density polyethylene

The PCL coated films had lower solubility in food simulants when compared to uncoated films (Table 21). The PCL coating prevented the direct contact of AH/ACNC with the food simulants, thereby reducing the dissolution of the films in the simulants. PCL is insoluble in food simulants

(Shen *et al.*, 2015), therefore, the solubilisation of the coated films in the simulants is evidence that the coating layer was porous. Furthermore, the SEM images, shown in (Table 22), support these findings, as pores can be seen on the surface of the AH/ACNC/PCL films. The reduced solubility of the film and porosity of the PCL layer can be exploited for development of AH/ACNC/PCL films with controlled and slow release of antioxidants.

Addition of polyphenols to the AH/ACNC/PCL films had no effect on the mechanical properties of the films ($p > 0.05$) but led to a reduction in the hydrophobicity of the films by 29.32% (Table 21). The increase in film hydrophilicity upon incorporating polyphenols may be due to the high water affinity of the mango polyphenols. In the presence of moisture, the hydroxyl groups of the polyphenols form hydrogen bonds with water molecules, thereby reducing the WCA of the films (López De Dicastillo *et al.*, 2011). The results by Fasihnia, Peighambardoust, Peighambardoust, & Oromiehie, (2018) are consistent with these findings when they reported a decline in water barrier properties of polypropylene films upon adding sorbic acid. Based on the results that the moisture barrier properties of the films were compromised by the inclusion of polyphenols, the AH/ACNC/PCL films are most suitable for packaging oily or fatty foods. Solid and semi-solid fatty foods have little or no moisture hence, a packaging with lower hydrophobicity may be suitable for containment of such food. However, the major drawback of using these films is that they may allow moisture from the environment to come into contact with the packaged fatty foods, thus promoting oxidation processes, and thereby compromising food shelf life (Samsudin *et al.*, 2014). However, the polyphenols in these films should be able to scavenge free radicals which facilitate the oxidation of the fatty foods and thus prevent food spoilage.

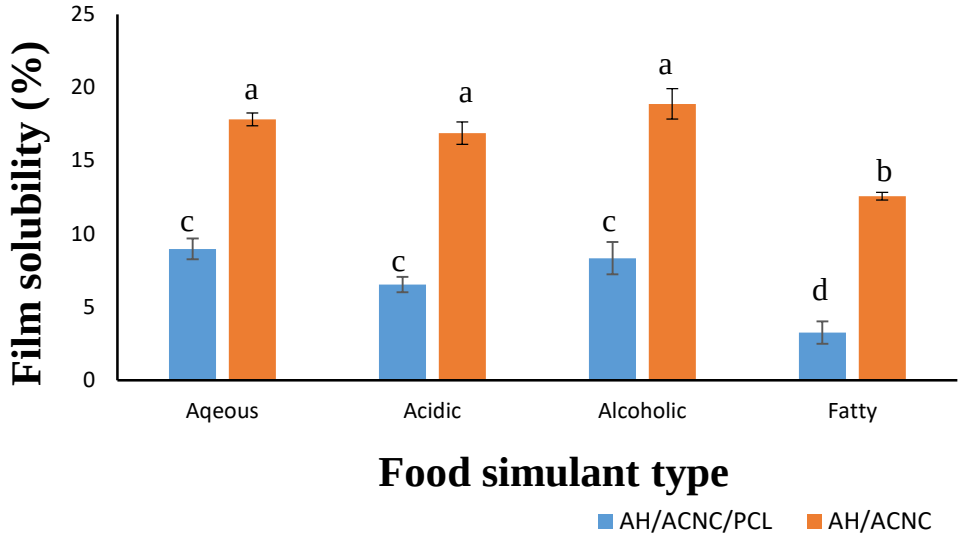


Figure 49: Solubility of acetylated hemicellulose-based films, with and without a coating of polycaprolactone, in food simulants. The values are means of three measurements. Values with different letters are significantly different ($p < 0.05$). The error bars represent standard deviation

6.17.5. Hemicellulose-based films as active food packaging material

Polyphenol release by the AH/ACNC/Polyphenol films into the food simulants depended on the food simulant type, time and temperature (Figure 50). The polyphenol content of all food simulants increased when temperature was increased from 5 ^{o}C to 40 ^{o}C and time from 0 h to 48 h. These results were in agreement with the antioxidant release profiles of polylactic acid films reported by Jamshidian & Tehrany, (2013) (Jamshidian *et al.*, 2013). A food packaging material capable of releasing antioxidants into food when food storage temperature deviates is important during food transportation. Normally, during transportation, food may be exposed to changes in temperatures and it could be hours before realising that food quality has been compromised (Biji *et al.*, 2015). Hence, an active packaging material capable of releasing antioxidants in response to temperature can minimise temperature dependent microbial growth and oxidation processes, thereby preventing food spoilage (Cesur *et al.*, 2017). The response of the AH/ACNC/Polyphenol/PCL films to temperature and time is an indication that these films may be suitable for utilisation as active food packaging material.

The rate of polyphenol release by the hemicellulose-based films (which ranged from 5.71 mg/h to 53.67 mg/h) was higher than that of LDPE films (which ranged from 0.0004 mg/h to 0.0041 mg/h) (Figure D1). The capability of hemicellulose-based films to release antioxidants in higher quantities in 2 days proves that these films have the potential to be used as active packaging material for short-term storage of food than LDPE films.

The highest polyphenol release by the films was into the fatty food simulant at 40 ^{o}C (Figure 50) followed by the alcoholic simulant and lastly the aqueous and acidic simulant. Fortunati *et al*. (2012) reported that there are two mechanisms responsible for antioxidant release by polymer matrices namely the swelling of polymers and solubilisation of antioxidants in the food simulant. The swelling of the polymer film when in contact with food simulants results in voids that act as passageways through which the antioxidants diffuse out of the film into the simulant, thus, promoting polyphenol release. In addition, the rate of release of polyphenols by polymers is boosted by the high solubility rates of polyphenols in the food simulant (Fortunati *et al.*, 2012). In this study, the highest polyphenol release was into the fatty simulant, a food media in which AH/ACNC/PCL films were least soluble (Figure 49). Furthermore, mango polyphenols are known to be highly soluble in ethanolic solutions, that is why these solutions are normally used for polyphenol extraction (Ajila, Bhat, *et al.*, 2007; Geerkens, Matejka, *et al.*, 2015). This implies that the higher solubility of the polyphenols in the fatty simulant (95% ethanol) than in the other simulants (with high water content 90-100%), was the major contributing factor to the release of

polyphenol by the films. Therefore, the AH/ACNC films prepared in this study are most suitable for packaging fat-rich foods.

6.17.5.1. Comparison of model predictions and experimental results of polyphenol release by acetylated hemicellulose-based films

The experimental and model data for polyphenol release by the hemicellulose-based films into food simulants and the resultant antioxidant activity of the food simulant are shown in Figure 50 and 51. The fitted curve model equations for the polyphenol release and antioxidant activity experimental data are highlighted in the Appendix section (Table D1 and Table D2). The polyphenol release by the AH/ACNC/Polyphenol/PCL films, into all simulants, in response to temperature and time, followed a logarithmic trend. The Migratest models were capable of predicting the release of mango polyphenols by the films into the food simulants within the first 5 h (Figure 50). The predicted polyphenol release deviated from the experimental results as the film-simulant contact time increased from 5 h to 48 h. The deviation can be due to the changes in the structure of the films as the polyphenols are released and partly because of the dissolution of the film matrix in the food simulants (Samsudin *et al.*, 2014). The structural changes are not accounted for during migration modelling using Migratest software, hence, the differences in the model predicted values and the experimental results.

Migration modelling using Migratest EXP software provides reliable predictions for the polyphenol release by the AH/ACNC/PCL films into the fatty food simulant, as shown by the close fitting of the model to the experimental data (Figure 50b). The model overestimated the release of the polyphenols from the films (Figure 50b and d) into the fatty and acidic simulant, which is a desirable outcome for theoretical estimation of polyphenol release. Several researchers have highlighted that theoretical migration models that overestimate the release of antioxidants into food are useful for the preliminary design of active food packaging to avoid exceeding the allowable antioxidant concentration limit for food (Gavriil *et al.*, 2018; Stärker and Welle, 2019). Underestimation of the polyphenol release by the models may result in the development of packaging material that releases antioxidants into food in concentrations higher than the allowable limit (Gavriil *et al.*, 2018; Stärker and Welle, 2019). The exposure of consumers to antioxidants higher than the allowable limit (60 mg/kg of food) can be a health hazard (Beigmohammadi *et al.*, 2016; EU, 2011; Peighambardoust, Beigmohammadi, & Peighambardoust, 2016). Both the AH based films (rate of release ranged from 0.0036 mg/kg to 0.043 mg/kg) and the LDPE based films (rate of release ranged from 0.013 mg/kg to 0.13 mg/kg) complied with the additives migration laws (Figure D1). Gavriil *et al.* (2018) highlighted that migration models should only be utilised for active packaging material design when the model predictions deviate less than 70% from the

experimental results. In this study, the least deviation of the Migratest predictions from experimental results was recorded for the fatty food simulant (< 66.4%) when compared to the other simulants. More importantly, the deviation of the models was less than 70%, providing a basis for using migration models for designing AH/ACNC based active packaging material for fatty food packaging. Notably, a logarithmic trend best describes (average R^2 of 0.87, Table D1) the experimental polyphenol release pattern over the release duration (Figure 50).

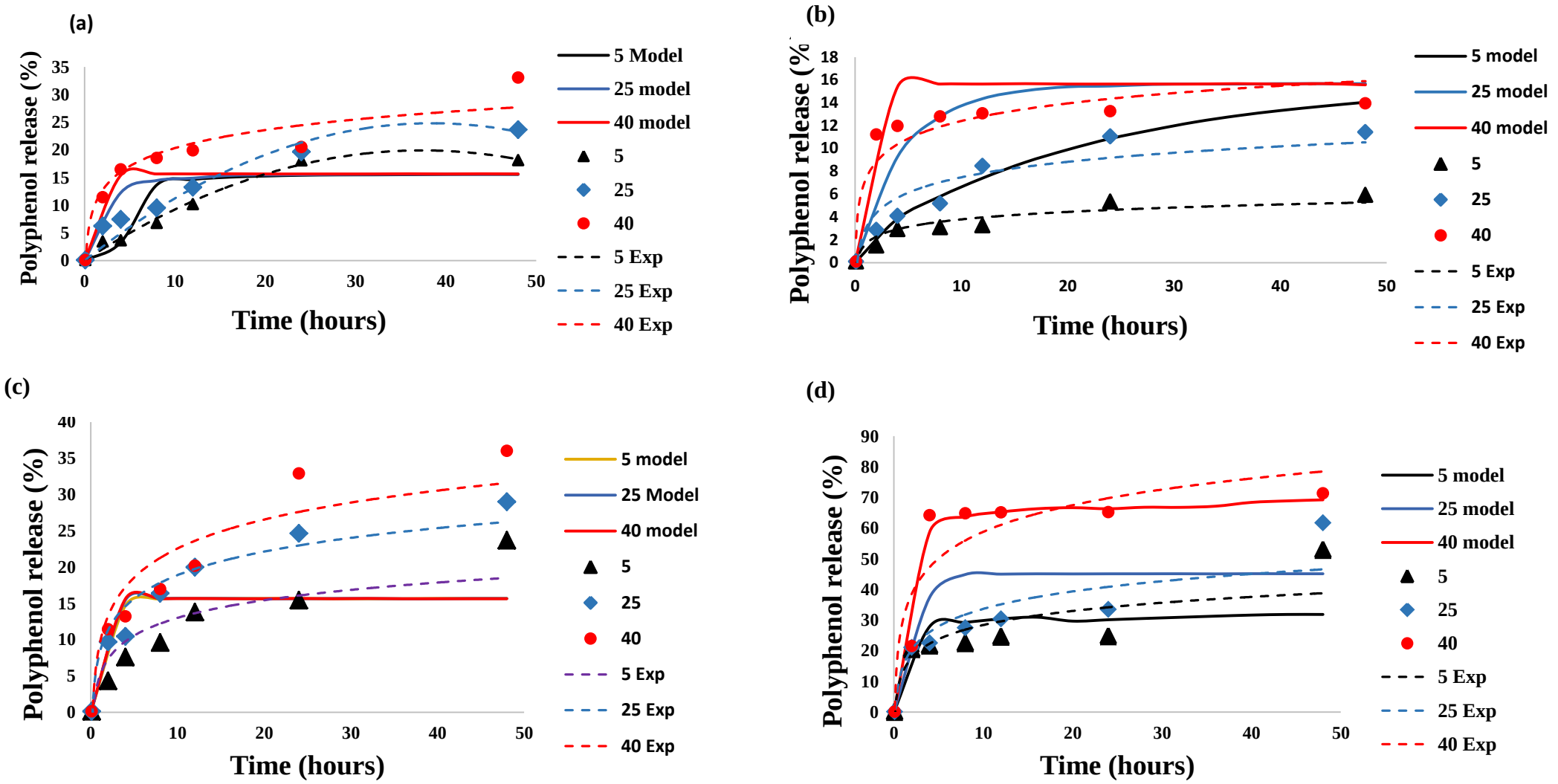


Figure 50: Effect of temperature and time on polyphenol release by the acetylated hemicellulose-based active packaging into the (a) aqueous, (b) acidic (c) alcoholic and (d) fatty food simulant. 5, 25, and 40 represent temperatures, in degrees Celsius, at which the experiments and modelling were done. The dots in the figure are the experimental results, the dashed lines are the fitted polynomial curves to the experimental data and the solid lines are the model predictions. Exp represents experimental results.

6.17.5.2. Antioxidant activity of the food simulants as affected by polyphenol release by the acetylated hemicellulose-based films

The antioxidant activity of the food simulants was dependent on temperature, time and type of simulant. The antioxidant activity increased with an increase in temperature from 5 ^{o}C to 40 ^{o}C in all the food simulants investigated (Figure 51). This observed phenomenon is crucial as free radicals that promote food spoilage tend to increase, especially for food stored at 5 ^{o}C, as temperature increases to 40 ^{o}C. Therefore, an increase in the antioxidant activity of the food, as temperature increases, minimises the quantity of free radicals in food, thereby reducing food spoilage (Malhotra *et al.*, 2015). The food simulant that had the highest antioxidant activity was the fatty food simulant at 40 ^{o}C. This could be because the highest release of polyphenols by the films was into the fatty food simulant (Figure 50) when compared to the other simulants. On the other hand, the antioxidant activity of the alcoholic, aqueous and acidic food simulants increased until a certain time and then decreased. The decline in the antioxidant activity of these simulants could suggest that there was interference on the antioxidant capabilities of the polyphenols in the simulant by co-migrated film material (Figure 51a) (Ribeiro *et al.*, 2008). The antioxidant profiles (Figure 51) were similar to those of the polyphenol release (Figure 50). by the AH/ACNC/PCL films, implying that there was a correlation between the polyphenol content of the simulant and antioxidant activity.

The antioxidant activity of the simulants exposed to the hemicellulose-based active packaging (10.97%-54.75%) had a higher antioxidant activity when compared to the simulants in contact with the LDPE-based films (0.12%-8.97%) (Figure 51 and D2). This is because of the capability of the hemicellulose films to release higher dosages of polyphenols into the simulant than the LDPE films (Figure D1). Therefore, the hemicellulose-based active packaging has the potential to be used as a replacement to LDPE films for short-term storage of food.

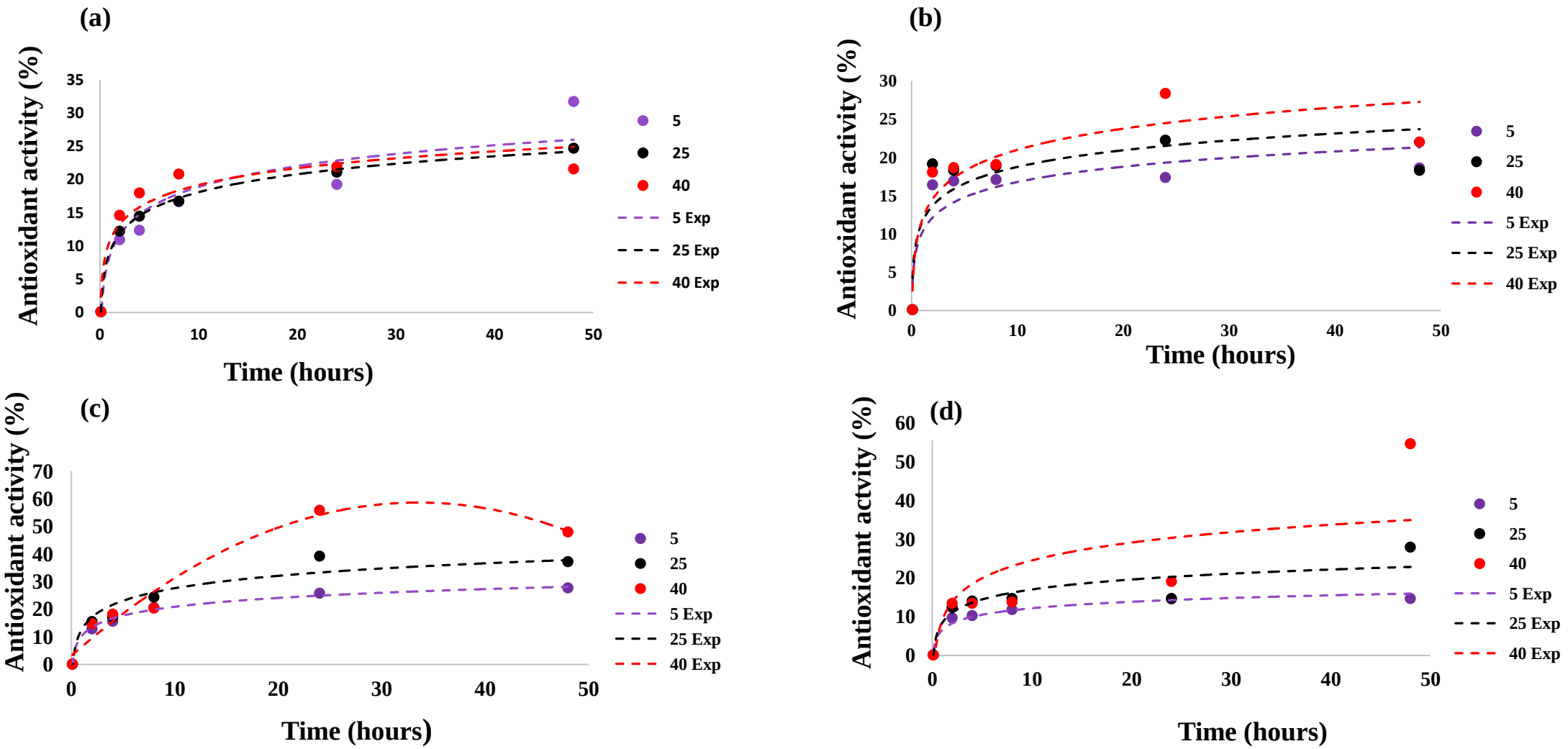
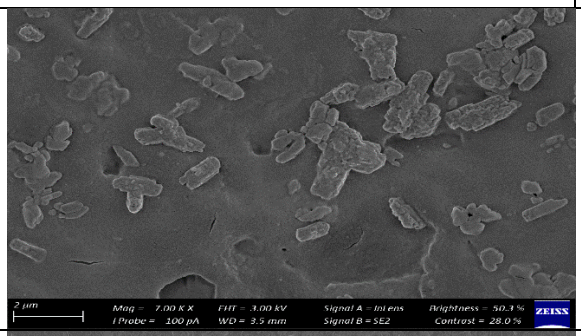
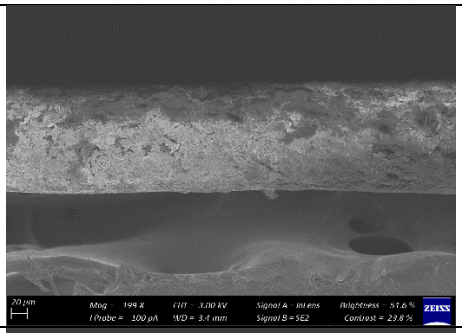
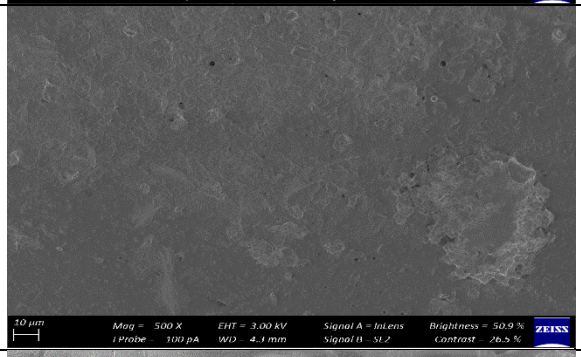
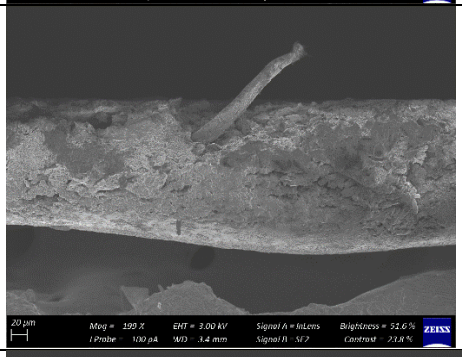
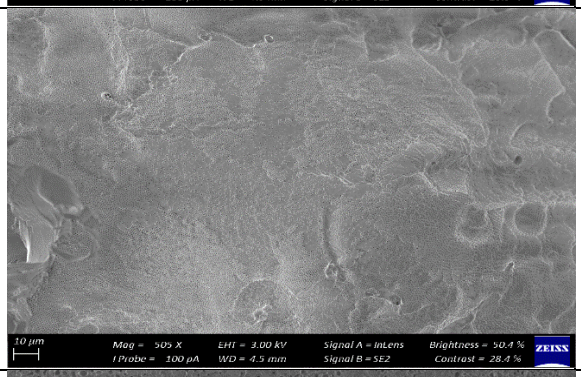
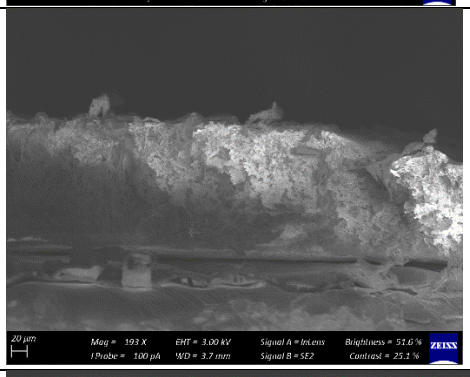
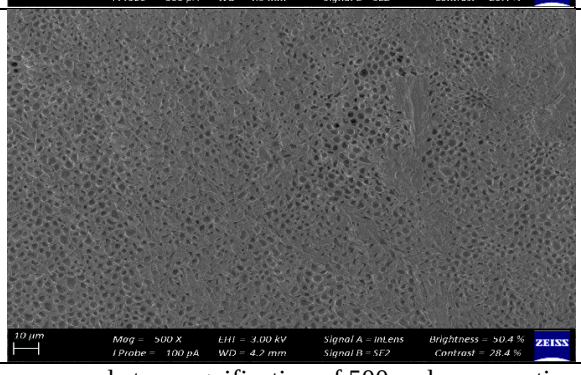
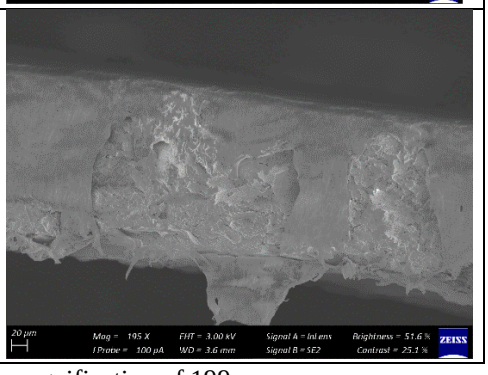


Figure 51: Antioxidant activity of the (a) aqueous, (b) acidic (c) alcoholic and (d) fatty food simulant in contact with acetylated hemicellulose-based active packaging as affected by temperature and time. 5, 25, and 40 represent temperature, in degrees Celsius, at which the experiments and modelling were done. The dots in the figure are the experimental results, the dashed lines are the fitted polynomial curves to the experimental data. Exp represents experimental results

6.17.6. Surface profiles of the acetylated hemicellulose-based films

Reinforcing AH films with ACNC resulted in a smoother film surface when compared to AH film with no reinforcement (Table 22). The effect could probably be because of the bonding of the AH matrix and the ACNC filler, forming a network which reduce the number of voids in the AH matrix (Dong *et al.*, 2017). However, the results reported by Dehghani, Peighambardoust, Peighambardoust, Hosseini, & Regenstein, (2019) contradicts these findings as they obtained rougher films after incorporating nanoparticles. Coating of the AH/ACNC films with PCL was successful as can be seen by the smooth surface profile of the AH/ACNC/PCL films. This shows that there were strong interactions between the coating and the AH/ACNC films (Gao *et al.*, 2018). However, integrating mango polyphenols in the AH/ACNC prior to coating with PCL led to the development of void spaces in the films as can be seen by the uneven surface profile of the AH/ACNC/PCL/Polyphenol films.

Table 22: Surface and cross-section morphology of acetylated hemicellulose-based films

Film	Film Surface	Film cross section
AH		
AH/ACNC		
AH/ACNC/PCL		
AH/ACNC/PCL/ Polyphenols		

SEM images of film surface measured at a magnification of 500 and cross section at a magnification of 199.
PCL: Polycaprolactone, AH: Acetylated hemicellulose, ACNC: Acetylated nanocellulose, LDPE: Low density polyethylene

6.17.7. Structural properties of acetylated hemicellulose-based films

The incorporation of ACNC into AH and coating AH/ACNC films with PCL, affected the structural properties of AH-based films (Figure 52). Particularly, the availability of hydroxyl groups in the AH films was affected.

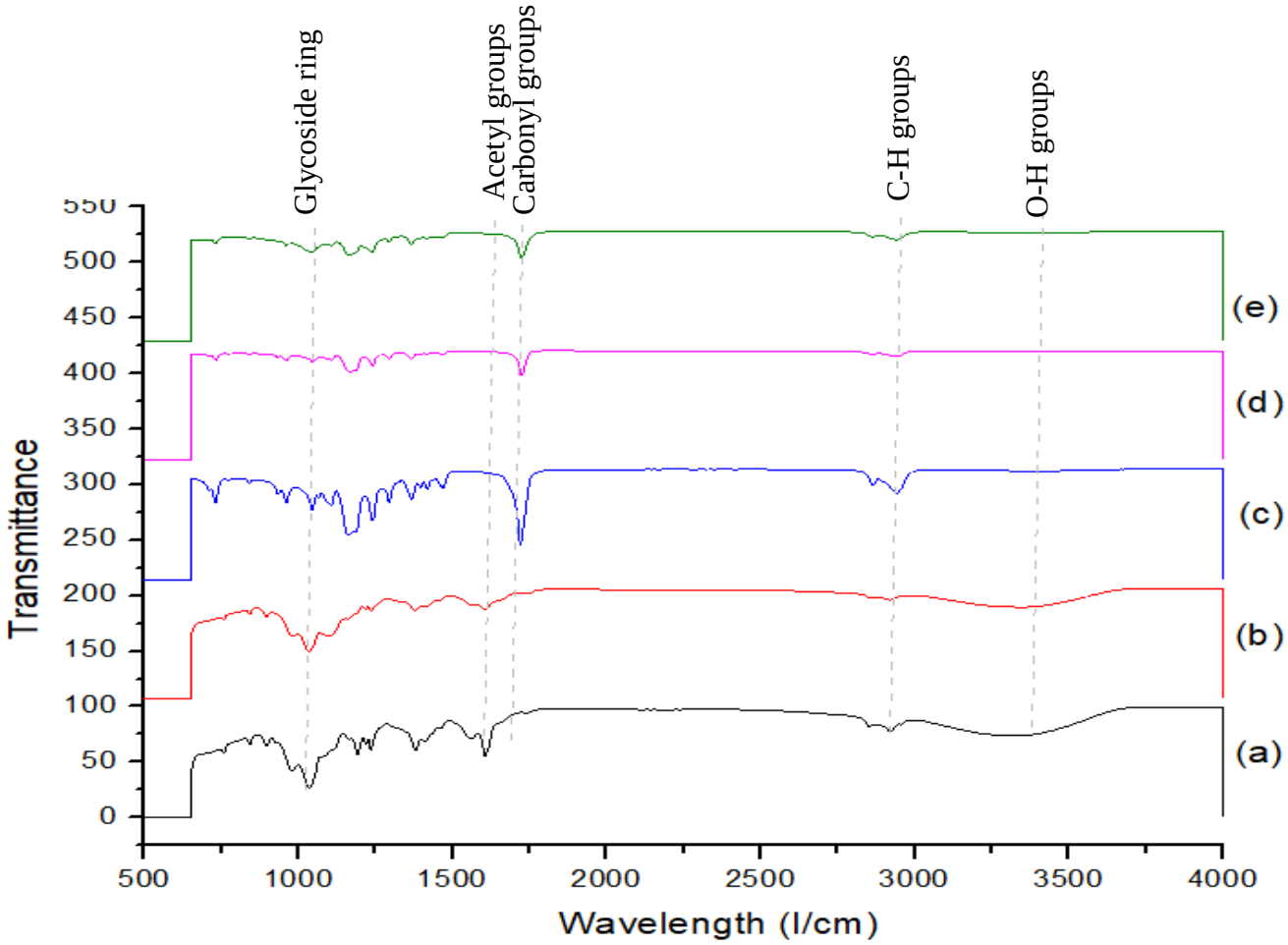


Figure 52: FTIR spectra of (a) acetylated hemicellulose film (b) Acetylated hemicellulose/nanocellulose film (c) Polycaprolactone film (d) Acetylated hemicellulose/nanocellulose film coated with polycaprolactone (e) Acetylated hemicellulose/nanocellulose film with mango polyphenols and coated with polycaprolactone

The O-H group band was reduced and disappeared when AH films (Figure 52) were reinforced with ACNC (Figure 52b) and coated with PCL, respectively (Figure 52d). These findings further confirms that reinforcing AH with ACNC and coating the films with PCL is a strategy for developing hydrophobic hemicellulose-based films.

6.17.8. Thermal stability of acetylated hemicellulose-based films

The thermal stability of AH–based films improved upon reinforcing the films with ACNC, incorporating mango peel polyphenols and coating the films with PCL. This is evident from Figure 53. T_{max} and $T_{5\%}$ of AH-based films increased with each modification step. Maximum weight loss was observed at 308 ^{o}C for AH films, 321 ^{o}C for AH/ACNC/PCL and 341 ^{o}C for AH/ACNC/PCL/Polyphenols. The temperature at which initial degradation of the AH-based films occurred increased from 91 ^{o}C to 106 ^{o}C with inclusion of ACNC and mango polyphenols and coating the films with PCL. Similar results were reported by researchers such as Gordibil *et al*., (2014) and Kadnam (2019). However, the AH films had a lower thermal stability to LDPE films

(Figure 53). The $T_{5\%}$ of the hemicellulose-based films was 106 ^{o}C, while that of LDPE films was 458 ^{o}C. Addition of mango polyphenols to LDPE films reduced the films' thermal stability slightly as highlighted by the reduction in $T_{5\%}$ by 2% (Figure D3). The hemicellulose-based films can be used for storing food at lower temperatures than the LDPE films. In addition, high temperatures may degrade the polyphenols in the hemicellulose-based films (Berardini, Knödler, *et al.*, 2005). Therefore, hemicellulose-based films can be used to substitute LDPE packaging used for storing food at low temperatures to reduce the degradation of packaging during storage.

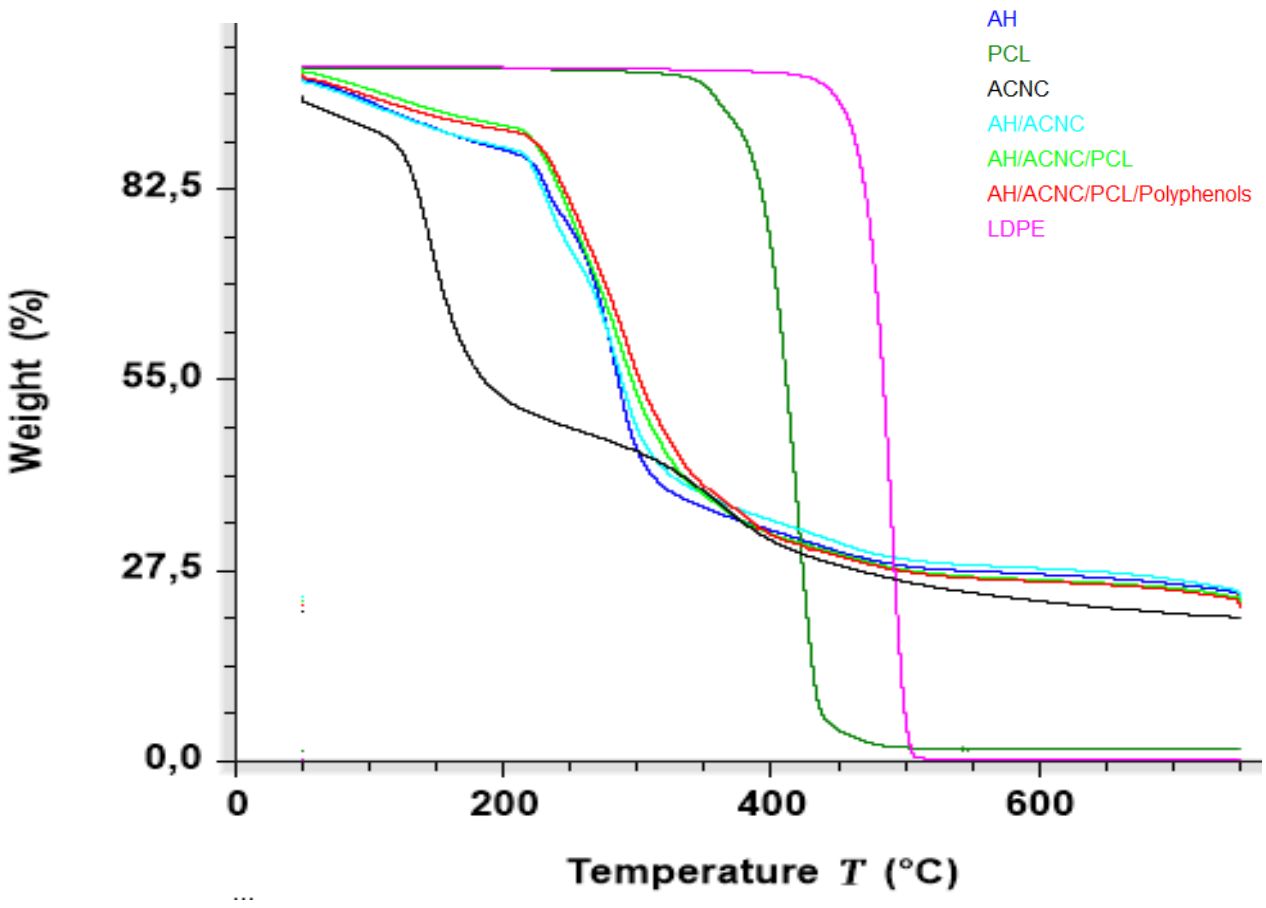


Figure 53: Thermal stability of acetylated hemicellulose-based films, low density polyethylene films and polycaprolactone films. AH:acetylated hemicellulose, PCL: polycaprolactone; ACNC: acetylated nanocellulose; LDPE:low density polyethylene

6.18. Conclusions

AH-based films' hydrophilicity and solubility in food simulants can be minimised by reinforcing the AH films with 50% ACNC of high DS (2.34) and coating the AH/ACNC films with PCL. The AH-based films were least soluble in the fatty food simulant when compared to the other food simulants, suggesting that their application should be for non-water-based systems and for storage in dry places. AH/ACNC/PCL films were able to encapsulate and release mango polyphenols into simulants in response to temperature and time. However, integrating polyphenols into the AH/ACNC/PCL films compromised some of the mechanical and functional properties of the films

such as tensile strength and moisture barrier capabilities respectively. Ficks' migration models are best suitable for predicting release of polyphenols, encapsulated in the AH films, into the fatty food simulant. Minimal dissolution of AH/ACNC films in the fatty simulant coupled with the ability to release polyphenols with detectable antioxidant activity into the fatty simulant, indicates that the films have potential application as active food packaging material for fatty foods. In addition, the film release of the bioactive substances into the fatty simulant can be modelled at design stage which can contribute towards cost effective design of packaging material.

7. UTILISATION OF MANGO ANTHOCYANINS AS A TEMPERATURE-TIME INDICATOR

Dissertation objectives fulfilled in this chapter

Objective 6 was fulfilled in this section. Anthocyanins recovered from mango peels can be utilised as temperature-time indicators. Anthocyanins colour change in response to changes in temperature and time was assessed with the aim of using anthocyanins as a temperature-time indicator.

Summary of findings

Objective 6 was successfully achieved as the anthocyanins showed a noticeable colour change with exposure to a temperature higher than 5 ^{o}C over two days. Therefore, mango anthocyanins have the potential to be used as temperature-time indicators.

Authors :Lindleen Mugwagwa, Annie Chimphango

Summary of authors' contributions

Lindleen Mugwagwa performed the experiments. Analysis and interpretation of results was a combined effort by the two authors. Lindleen did 80% of the work in this chapter and Annie contributed 20% of the work.

7.1. Introduction

There has been a recent increase in the formulation of freshness indicators from non-toxic, biodegradable raw materials to replace indicators derived from non-renewable synthetic feedstock (Shukla *et al.*, 2016; Balbinot-alfaro *et al.*, 2019). Temperature-time indicators have found use in the food industry with the aim of minimising health hazards associated with improper storage of food (Maciel *et al.*, 2012). These indicators show when food storage temperature deviates from the optimum and also when food has passed its expiry date. Temperatures beyond the recommended food storage temperature facilitate reactions that cause food spoilage, thereby compromising food quality. Anthocyanins are among the chemicals which have been tested as indicators due to their ability to change colour in response to temperature and time (temperature-time indicator). The colour change of anthocyanins is greatly influenced by the source, type of anthocyanin and quantity of anthocyanins (Gould *et al.*, 2008). Anthocyanins which have been used in developing temperature-time indicators were extracted from the red cabbage and plums (Xavier *et al.*, 2008; Shukla *et al.*, 2016). However, utilisation of anthocyanins from the aforementioned sources to

develop temperature-time indicators competes with food sources. Peels from the mango fruit, which are otherwise discarded as waste during the mango processing industry, are a potential source of anthocyanins. Previous research has only focused on quantifying mango peel anthocyanins without evaluating their application as temperature-time indicators (Berardini, Fezer, *et al.*, 2005; Masibo and Qian, 2008). Therefore, this study aimed at assessing the utilisation of mango peel anthocyanins as temperature-time indicators in storage conditions used for food storage (0 ^{o}C, 5 ^{o}C, 25 ^{o}C and 40 ^{o}C). That is, the effect of temperature and time on the colour change of mango peel anthocyanins was studied.

7.2. Materials and methods

Sodium hydrogen phosphate was supplied by Sigma Aldrich, Germany.

7.1.1. Development of the temperature time indicator

Anthocyanin extraction from mango peels and analysis was carried out according to the method in Chapter 4, section 4.10. To develop the temperature-time indicator approximately 1 mL of the anthocyanins extract (38.8 mg/100 g) was diluted with 12 mL of sodium hydrogen phosphate buffer at a pH of 7 (Maciel *et al.*, 2014). The mixtures were contained in stoppered glass bottles and incubated at 0 ^{o}C, 5 ^{o}C 25 ^{o}C and 40 ^{o}C for three days .

7.1.2. Anthocyanin colour measurements

The anthocyanin colour measurements were carried out using a Color-guide glass b 45/0 chroma meter (Gardner, BYK, Germany). The colour measurements were done for a period of three days after exposing the anthocyanins to temperature ranges of 0^{o}C, 5 ^{o}C 25 ^{o}C and 40 ^{o}C. The chroma meter was calibrated against a white plate and colour measurements were done with a CIE (L, a, b) system. L represents lightness ranked from 0 (opaque) to 100 (to transparent), a negative **a** value represents redness in the sample and greenness is represented by a positive **a** value. The letter **b** symbolises yellowness when a positive value is obtained and blueness when a negative value is obtained. Colour measurements were performed in triplicate.

The colour tone, Hue (H^{0}) was calculated based on the Equation 48 below

$$\text{Colour tone} = \tan^{-1}\left(\frac{\boldsymbol{b}}{\boldsymbol{a}}\right) \qquad (48)$$

And the colour intensity was obtained based on Equation 49 below

$$\text{Colour intensity} = (\boldsymbol{a}^2 + \boldsymbol{b}^2)^{0.5} \qquad (49)$$

7.3. Results and discussions

The colour intensity of mango anthocyanins increased with an increase in temperature and time (Figure 54a) The anthocyanins extract lost its lightness and became more opaque with an increase

in time and temperature as shown by a reduction in the lightness parameter in Figure 54b. This is attributed to the degradation of anthocyanins with time and also with extended exposure to high temperatures. Hue values, which depict the actual colour of the extract, in this case decreased with time showing the changes in colour of anthocyanins from yellow to green. The relatively lower changes in the colour parameters when the anthocyanins were stored at 0 ^{o}C and at 5 ^{o}C is evidence that anthocyanins are stable at lower temperatures. Higher temperatures facilitated their degradation which is shown by a rapid change in colour parameters as the temperature increased from 0 ^{o}C to 40 ^{o}C. West *et al.* (2013) reported that a temperature increase up to 35 ^{o}C degrades up to half of the initial anthocyanins content (West and Mauer, 2013). Therefore, taking advantage of these properties, a temperature-time indicator was developed in this study. As can be seen in Figure 55, there was a distinct and noticable difference in the colour of the anthocyanin temperature-time indicator when the indicator was stored at 5 ^{o}C and at 40 ^{o}C for 3 days. Therefore, the colour of the indicator would change when food which is supposed to be stored at 5 ^{o}C is exposed to higher temperatures. It is worth noting that the anthocyanin colour change was not reversible when the indicator was exposed to 40 ^{o}C, then later stored at lower temperatures. Mango anthocyanins have demonstrated potential to be utilised as a temperature-time indicator for frozen food or food that has to be stored at 5 ^{o}C. When such foods are exposed to higher temperatures a colour change in the anthocyanins will be seen.

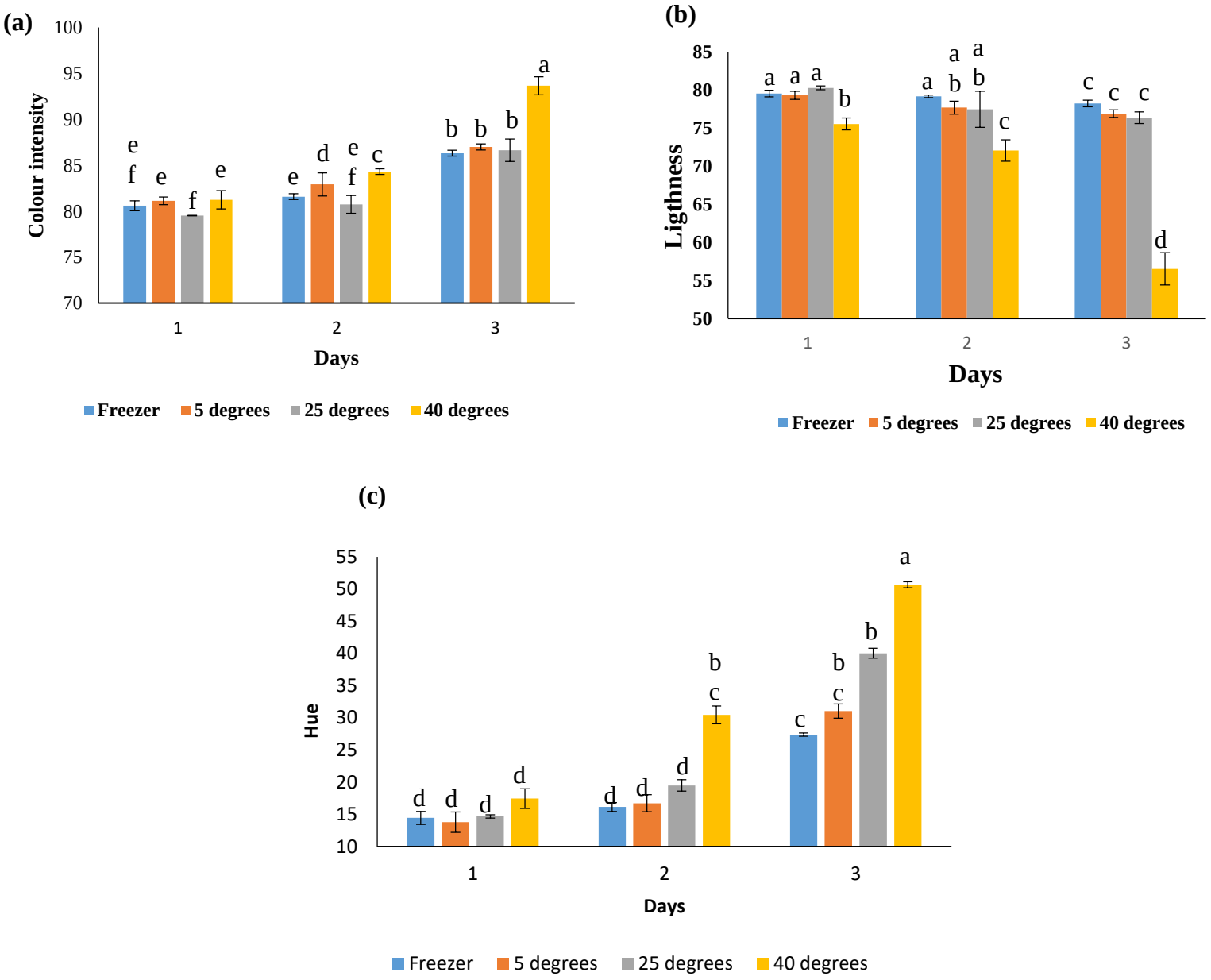


Figure 54: Colour intensity (a), Tone (b) and Hue (c) for anthocyanin extracts at different temperatures over 3 days. The values are means of three measurements. Values with different letters are significantly different ($p < 0.05$). The error bars represent standard deviation

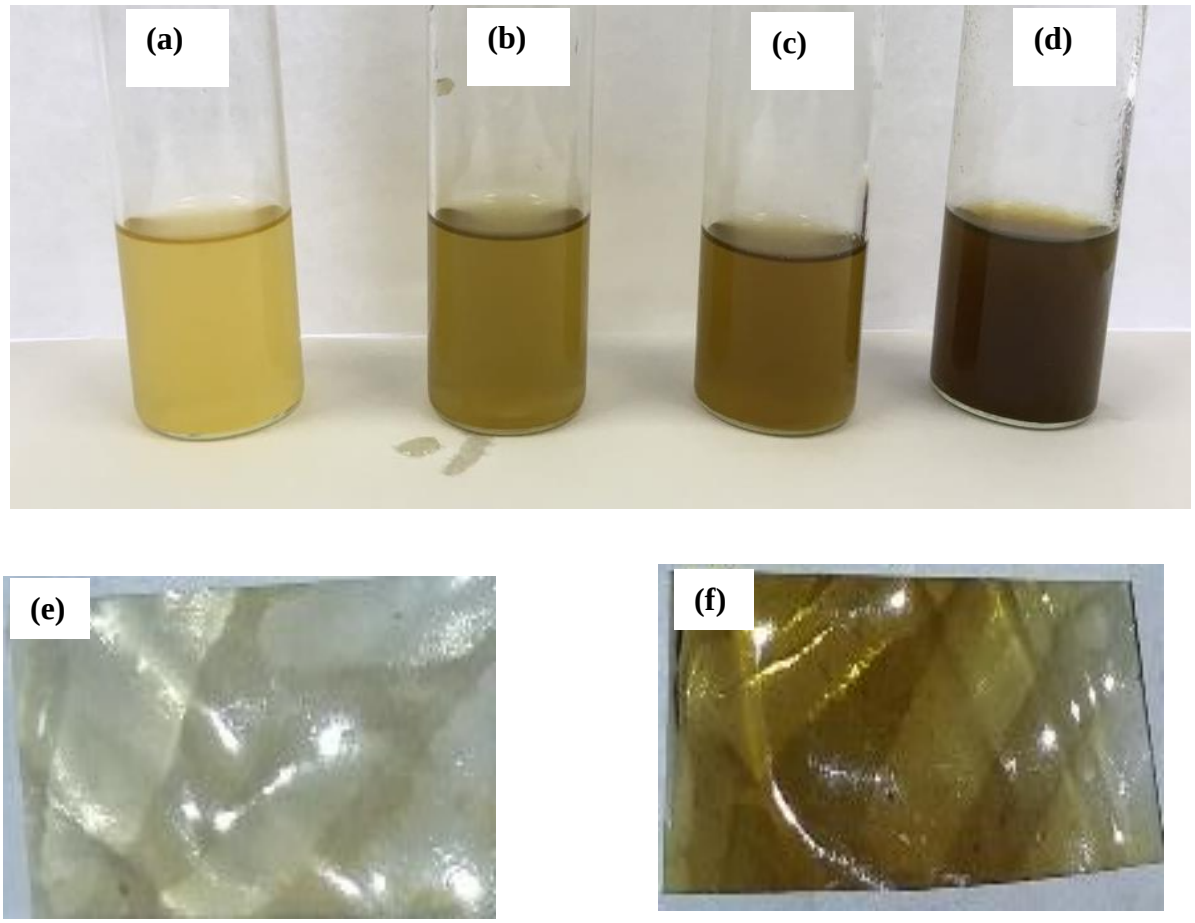


Figure 55: Colour change of anthocyanin extracts in response to temperature and time: (a) reference (b) 40 °C,1 day, (c) 40 °C, 2 days (d) 40 °C, 3 days e) 5 °C, 3 days (f) 40 °C , 3 days

8. General discussion, conclusion and recommendations

8.1. General discussion

The research involved developing processes for acquiring and modifying by-products (biopolymers and bioactive compounds) from agricultural residues, and evaluating the active food packaging material formulated from integrating these agro-derivatives. The development and application of hemicellulose-based films as alternative active food packaging films to LDPE films was investigated. In addition, a temperature-time indicator was developed and its potential application in a food environment was assessed. The following section provides a general discussion of the findings of the study.

8.1.1. Fractionation of agricultural residues to recover biopolymers and bioactive compounds

Biopolymers (hemicellulose and pectin) and bioactive compounds (anthocyanins and polyphenols) which were used in developing the active packaging and temperature-time indicator were extracted from wheat straw and mango peels. Hemicellulose was extracted from wheat straw after pre-treating the wheat straw with an ethanol/NaOH mixture. The alkali-catalysed organosolv pre-treatment of wheat straw before hemicellulose extraction was optimised with the aim of enhancing hemicellulose hydrophobicity, modification by acetylation and hemicellulose-filler compatibility. Hemicellulose with varying composition can be obtained after applying different pre-treatment conditions to biomass (Jeong *et al.*, 2010; Borand and Karaosmanoğlu, 2018). Manipulating biomass pre-treatment methods can be a strategy to effectively modify the composition of hemicellulose to suit a certain application prior to its extraction. Therefore, optimising biomass pre-treatment methods has the potential to tailor-make the composition of the hemicellulose to enable effective hemicellulose modification by processes such as acetylation, and improving its hydrophobicity and interaction with fillers. The alkali-organosolv based pre-treatment of wheat straw was observed to significantly ($p < 0.05$) affect hemicellulose modification by acetylation, and in turn, the mechanical and water resistivity of the hemicellulose-based films (Chapter 5). However, the focus of previous research has been on optimising biomass fractionation methods to increase hemicellulose yields and to determine the effect of the extraction conditions on the hemicellulose composition. In addition, biomass pre-treatment methods have been optimised with the aim of enhancing enzyme-based fermentation of hemicellulose (Mesa *et al.*, 2011; Diner and Fan, 2012). However, the manipulation of biomass pre-treatment methods to boost acetylation,

hemicellulose-filler interaction and hydrophobicity is lacking. This information is particularly important when aiming at improving the water-barrier and mechanical properties of hemicellulose-based films.

Methods that have previously been used to improve the hydrophobicity of hemicellulose-based films are chemical-based processes (such as acetylation) and enzyme-based processes. In these processes side branches such as arabinose and uronic acids were cleaved or hydroxyl groups in the side chains were substituted with hydrophobic groups (acyl groups) (Sternemalm *et al.*, 2008; Belmokaddem *et al.*, 2011). The results in Chapter 5 indicate that a combination of the aforementioned phenomenon is more effective in enhancing hemicellulose hydrophobicity than applying only one of the modification technologies. The side chains can be manipulated during biomass pre-treatment before extracting hemicellulose, thereby eliminating the need for the costly enzymatic cleaving process after hemicellulose extraction. The cleaving of the side chains during biomass pre-treatment prior to hemicellulose extraction to boost the replacement of hydroxyl groups in hemicellulose with hydrophobic acyl groups during acetylation, has not been evaluated. On the other hand, hemicellulose hydrophobicity is attributed to the degree of acetylation alone, without taking into consideration the hemicellulose composition (Zhou *et al.*, 2013). The findings in Chapter 5, however, showed that the hemicellulose composition had a greater impact on hydrophobicity than the degree of acetylation. In fact the pre-treatment reduced the acetylation degree of substitution required for hemicellulose to exhibit high water resistivity. Hence, pre-treatment of biomass can reduce the extent of downstream processing of hemicellulose required to achieve hydrophobicity. On the other hand the pre-treatment conditions that favoured acetylation boosted hemicellulose-filler compatibility and the mechanical properties of hemicellulose-based films. Therefore, this study provided evidence that downstream processing of hemicellulose can be enhanced by manipulating the biomass fractionation methods prior to hemicellulose recovery. The downstream processing, in turn, affects the end-application of the hemicellulose. In order to develop hemicellulose-based films suitable for food packaging, it is therefore, necessary to control the conditions applied during the hemicellulose recovery stage, taking into consideration downstream hemicellulose modification processes and properties required in a packaging material.

Anthocyanins, polyphenols, and pectin were recovered from mango peels. The sequential extraction of functional biopolymers and bioactive compounds is hindered by the differences in optimum extraction conditions of the products, resulting in low yields or loss of functional properties. A three-stage sequential extraction process was developed and optimised to recover anthocyanins, polyphenols, and pectin from mango peels. The effects of each extraction stage on the recovery of downstream products were determined. The results highlighted in Chapter 4 suggest

that the three-stage sequential extraction process recovers functional anthocyanins, polyphenols, and pectin that can be used in developing active food packaging films and temperature-time indicators. Therefore, biopolymers and bioactive compounds, which have properties required in developing active food packaging and temperature-time indicators, can be recovered from the same biomass. Sequential extraction of biopolymers and bioactive compounds from mango peels provides a basis for the development of mango waste biorefineries. In addition, the multi-stage process is a step towards fully exploiting underutilised fruit waste.

8.1.2. Development of hemicellulose-based active food packaging material

Hemicellulose-based active packaging films, AH/ACNC/Polyphenol/PCL and hemicellulose/pectin/nanocellulose films (Chapter 6) were developed by solvent casting. The hemicellulose-based films' mechanical properties, solubility in simulants and antioxidant release into food simulants were evaluated using LDPE films as benchmark. The application of hemicellulose-based films as active packaging is still lacking due to the high solubility of these films in aqueous solutions. In addition, despite the fact that unmodified hemicellulose (no acetylation), pectin and nanocellulose are not soluble in ethanol (ethanol is used as a fatty food simulant), biocomposite films developed from blending these biopolymers have not yet been formulated and tested as a packaging material for fatty foods (Koubala, Kansci, *et al.*, 2008; Peng, Peng, Xu and Sun, 2012). The findings in Chapter 6 present a basis for utilising the hemicellulose/pectin/nanocellulose biocomposite films as active packaging material for fatty foods. However, these films are restricted to the fatty foods (5% water content), as they completely disintegrate in the other foods such as acidic, aqueous and alcoholic foods, due to high water content of these food types (> 90% water content) when compared to the fatty simulant. In order to increase the application of hemicellulose films as packaging material for food with higher water content, hemicellulose can be modified by acetylation, reinforced with hydrophobic fillers and coated with hydrophobic material.

Although acetylation and reinforcing AH with ACNC has been proposed by several researchers to increase the WCA of the films, the films have not been tested as food packaging for wet food. In addition, the ACNC that was used as reinforcement for AH-based films had a low degree of acetylation. This could have been because information was lacking on the dispersibility and compatibility of ACNC with higher DS with AH. Therefore, the results in Chapter 6 show that hemicellulose can be effectively modified by acetylation, reinforced with ACNC of high DS and coated with PCL to form films with similar mechanical properties and hydrophobicity to LDPE films. Furthermore, the aforementioned modifications enhanced the stability of the hemicellulose-based films when in contact with different food simulants.

The ability of AH films to encapsulate and release antioxidants into food simulants leading to an increase in the antioxidant activity of the simulants in response to temperature and time provides a basis for utilising hemicellulose-based films as active food packaging films. Hemicellulose-based films have proven to release higher dosages of antioxidants, that have high antioxidant activity, into food simulants within a shorter time-frame as temperature and time increased when compared to LDPE films. Therefore, hemicellulose-based active packaging films have potential to substitute for the LDPE active films used for packaging perishable foods. This work diversifies and increase the utilisation of hemicellulose as a packaging material capable of manipulating packaged food to increase shelf life.

Models are commonly utilised during the design stage of packaging material to predict the performance and properties of packaging material. The application of models to predict the mechanical properties and antioxidant release by hemicellulose-based films was evaluated in Chapter 6. The micromechanical models that are suitable for predicting the mechanical properties of the hemicellulose-based films are the Tsai-Pagano model (Young's modulus) and the Bowyer-Bader model (tensile strength). The results in Chapter 6 indicate that the use of Migratest software for estimating antioxidant release from the hemicellulose-based films gives the most accurate results when the packaging film is utilised for packaging fatty foods. Therefore, the mechanical properties and antioxidant release of the hemicellulose-based films adhere to theoretical principles used in designing food packaging material.

8.1.3. Development of a temperature-time indicator

A temperature-time indicator was developed from the mango anthocyanins. During food storage or transportation, food can be exposed to undesirable temperatures which can result in the accumulation of harmful microorganisms. Food shelf life and the health of consumers can be compromised by the fluctuation in food storage temperature (Manohar *et al.*, 2015). Most perishable food is stored at low temperatures and the food quality changes when temperature increases. Therefore, there is a need for an indicator to show if the food storage temperature has at any point deviated from the optimal. The application of mango anthocyanins, as a temperature-time indicator has not been studied as research has only focused on quantifying anthocyanins in mango peels. The results in Chapter 7 have proven that mango anthocyanins can be used as a temperature-time indicator as the anthocyanins irreversibly changed colour in response to temperature and time.

8.2 Conclusions

Biopolymers and bioactive compounds extracted from lignocellulosic biomass can be exploited as alternative raw material for formulating active food packaging and temperature time indicators. However, the coexistence of biopolymers, bioactive compounds and other plant material affects their manipulation during biomass fractionation. The application of biopolymers in developing active packaging material to replace LDPE based films is limited by the hydrophilicity and poor mechanical properties of the biobased films. Therefore, there is a need to develop processes for recovering functional biopolymers and bioactive compounds and modifying them with the aim of developing an active packaging using LDPE as benchmark.

In this study, alkali-based organosolv pre-treatment of wheat straw was optimised to enhance the hemicellulose modification by acetylation, AH compatibility with ACNC and subsequently the mechanical properties and moisture barrier properties of the AH films. The alkali-based organosolv pre-treatment conditions had a significant effect on the hemicellulose composition, the acetylation process, hydrophobicity and mechanical properties of the AH films. The manipulation of pre-treatment conditions of wheat straw has proven to be a move towards enhancing the application of hemicellulose-based films as food packaging material. Therefore, in order to formulate hemicellulose-based films with improved water barrier properties, the hemicellulose recovery methods should be controlled to instill water resistivity into the hemicellulose during fractionation, thereby reducing the extent of acetylation required. In addition, the alkali-catalysed pre-treatment process has proven to improve the reinforcing efficiency of ACNC in AH films. The effectiveness of biopolymer modification processes such as acetylation depend on the biomass pre-treatment methods utilised prior to biopolymer extraction.

In order to determine the effects of nanocellulose DS and loading on the AH films physicochemical properties, nanocellulose was acetylated to DS ranging from 0 to 2.34 and the ACNC loading in AH films increased from 10% to 50%. The study showed that increasing the DS of nanocellulose enhanced the barrier, mechanical properties and reduced the solubility of the reinforced AH-based films in food simulants. Furthermore, the reinforcement efficiency of ACNC in AH can be improved by increasing ACNC loading from 10% to 50%. The effect of a PCL coating on the AH/ACNC films' mechanical properties, hydrophobicity and solubility in food simulants was evaluated. The coating further improved the mechanical properties, hydrophobicity and stability of the AH-based films when in contact with food simulants. There was no significant difference between the hydrophobicity and mechanical properties of the AH based films and that of LDPE films. The Bowyer-Bader model was the best micromechanical model for predicting the AH-based films tensile strength while the Cox-Krenchel accurately predicted the films' Young's

modulus. Therefore, this study proved that AH films require ACNC with high DS and loading as reinforcement and a coating of PCL to develop food packaging material to replace LDPE films.

Anthocyanin, polyphenols, and pectin can be successfully extracted from mango peels, using a three-stage sequential extraction process, without compromising the functional properties of the products. In fact, the sequential extraction improves the purity of the pectin. Biopolymers and bioactive compounds can therefore be extracted sequentially from the same biomass and still maintain functional properties required in active packaging material and temperature-time indicators.

Active packaging material was formulated by incorporating polyphenols and pectin in hemicellulose-based films. The polyphenol release by the hemicellulose-based films into food simulants was observed to increase when incubation temperature and time increased. Utilisation of migration models to predict the polyphenol release by the hemicellulose-based films was found to be more suitable for the films which were in contact the fatty foods when compared to the other simulants investigated. Hemicellulose-based films are capable of releasing almost all the encapsulated antioxidants into food at a faster rate than LDPE films within two days. Therefore, the films have potential application as active packaging material to increase the shelf life of perishable foods.

Mango anthocyanins were able to show colour changes in response to temperature overtime. Therefore, they can be used as temperature-time indicators in the food industry to show when food has been exposed to undesirable temperatures.

8.3 Recommendations

This work highlighted the potential utilisation of hemicellulose-based films as active food packaging material. The hemicellulose-based films were evaluated for their barrier properties and migration into food, using food simulants. It is recommended that the stability of the hemicellulose-based films when in contact with actual food be studied. In addition, the application of the methods developed in this study to other agricultural residues, is required to increase the sources of biopolymers and bioactive compounds that can be utilised in developing active food packaging.

The AH-based films developed in this research had no plasticizers, hence, they were less flexible when compared to LDPE films. Therefore, the incorporation of plasticizers into these films, with the aim of improving the flexibility of the films is a subject for further study. In addition, the films were developed by solvent casting at laboratory scale, therefore, upscaling the processes to pilot scale and large scale, is required. A techno-economic evaluation and Life cycle assessment of the

processes used in developing the hemicellulose-based films, is required to determine the sustainability of the films.

9. References

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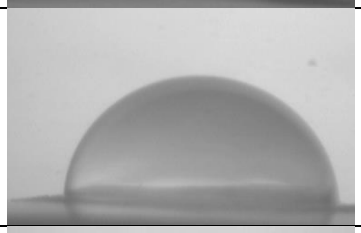
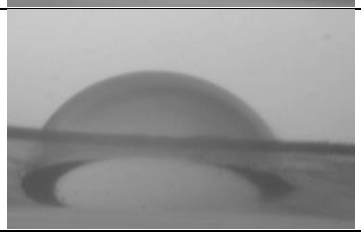
Appendix A: Hydrophobicity and surface profile of acetylated hemicellulose-based films

Table A1: Water contact angle images for the hemicelluloses extracted after organosolv pre-treatment of wheat straw

Run	Water contact angle image
1	
2	
3	
4	
5	
6	
7	

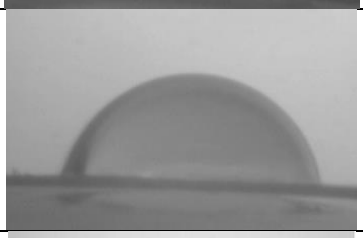
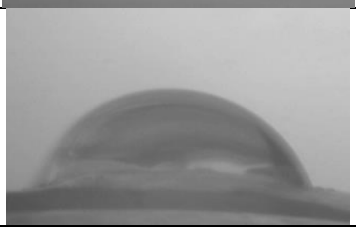
8	
9	
10	
11	
12	
13	
14	
15	

Table A2: Water contact angle images for undelignified hemicellulose and hemicellulose from the optimum conditions.

Sample	Water contact angle image
Undelignified hemicellulose	
OPT 1 hemicellulose	
OPT2 Hemicellulose	

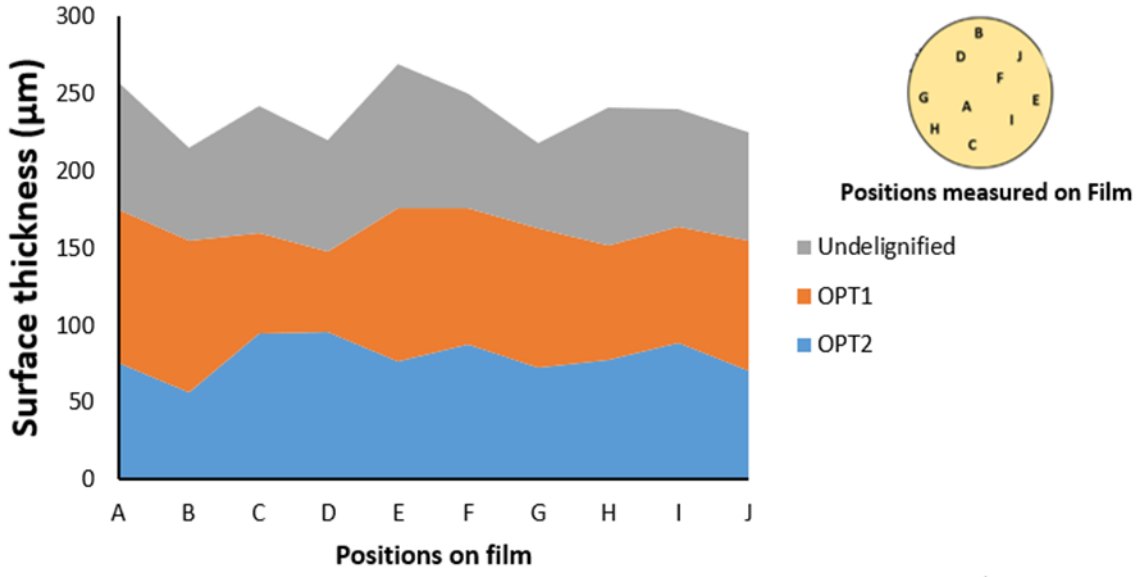


Figure A1: Surface profile of the hemicellulose films developed from the hemicellulose extracted at optimum conditions and from unpre-treated wheat straw. OPT; Optimum

Appendix B: Physico-chemical properties and antioxidant release by hemicellulose/pectin/nanocellulose films

Table B1: Composition of hemicellulose used for developing hemicellulose/pectin/nanocellulose films

Uronic acid content (%)	Lignin content (%)	Xylose content (%)	Arabinose/xylose ratio	Molecular weight (g/mol)
8.91	4.6	80.3	0.087	39 221

Table B2: Composition of pectin used for developing hemicellulose/pectin/nanocellulose films

Uronic acid content (%)	Neutral sugars content (%)	Molecular weight (g/mol)
85.5	43.6	352 879

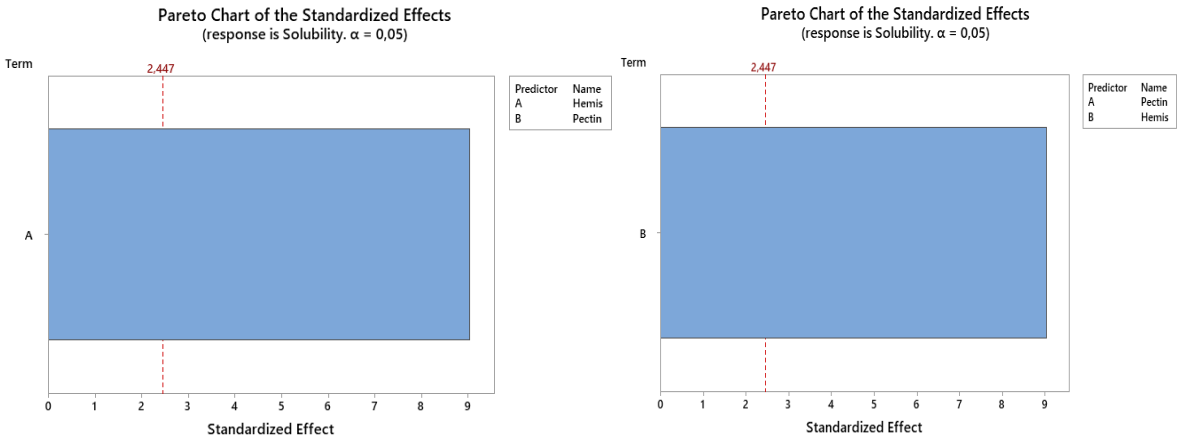


Fig. B1: Estimation of the effects of hemicellulose and pectin composition of films on film solubility in the fatty food simulant

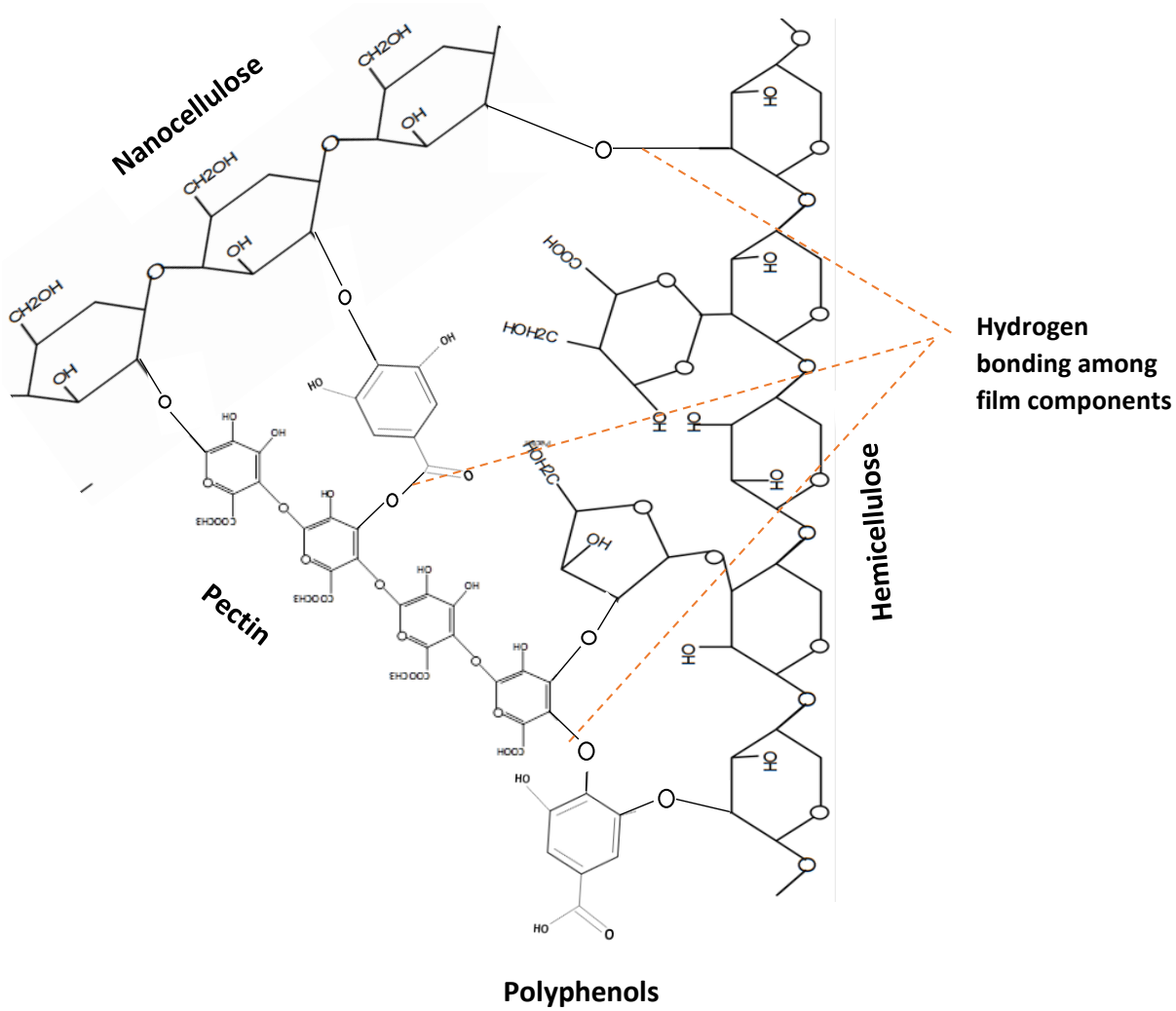


Fig. B2: Typical bonding in a film with hemicellulose, pectin and polyphenols

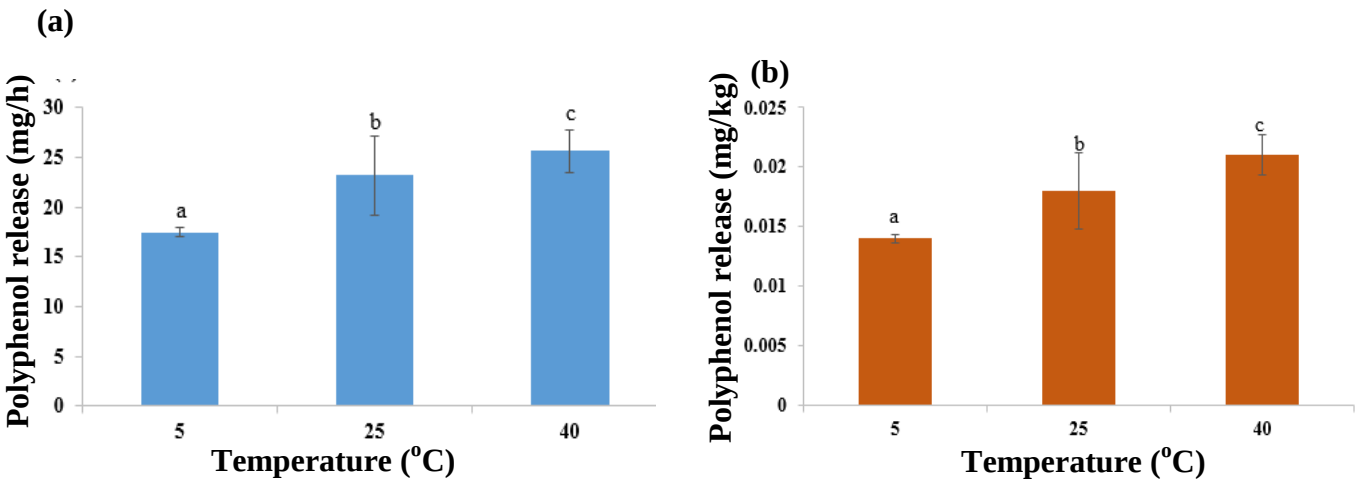


Fig. B3: Hemicellulose/pectin/nanocellulose films' rate of polyphenol release into the fatty food simulant in (a) mg/h and (b) mg/kg of food simulant

Table B3: Models and R^2 values for polyphenol release from hemicellulose based films into food simulants

Simulant type	Temperature (^oC)	Model	R^2 Value
Fatty	5	$9.629\ln(x) + 16.586$	0.8708
	25	$13.912\ln(x) + 25.454$	0.9245
	40	$15.258\ln(x) + 29.181$	0.954

Models were developed using Statistica version 7, 13.2 software

Table B4: Models and R^2 values for antioxidant activity of simulants in contact with hemicellulose films

Simulant type	Temperature (^oC)	Model	R^2 Value
Fatty	5	$3.2056\ln(x) + 7.3712$	0.9395
	25	$2.782\ln(x) + 6.8314$	0.9626
	40	$3.2056\ln(x) + 7.3712$	0.9395

Models were developed using Statistica version 7, 13.2 software

Appendix C: Dispersion of acetylated nanocellulose in acetylated hemicellulose-based films

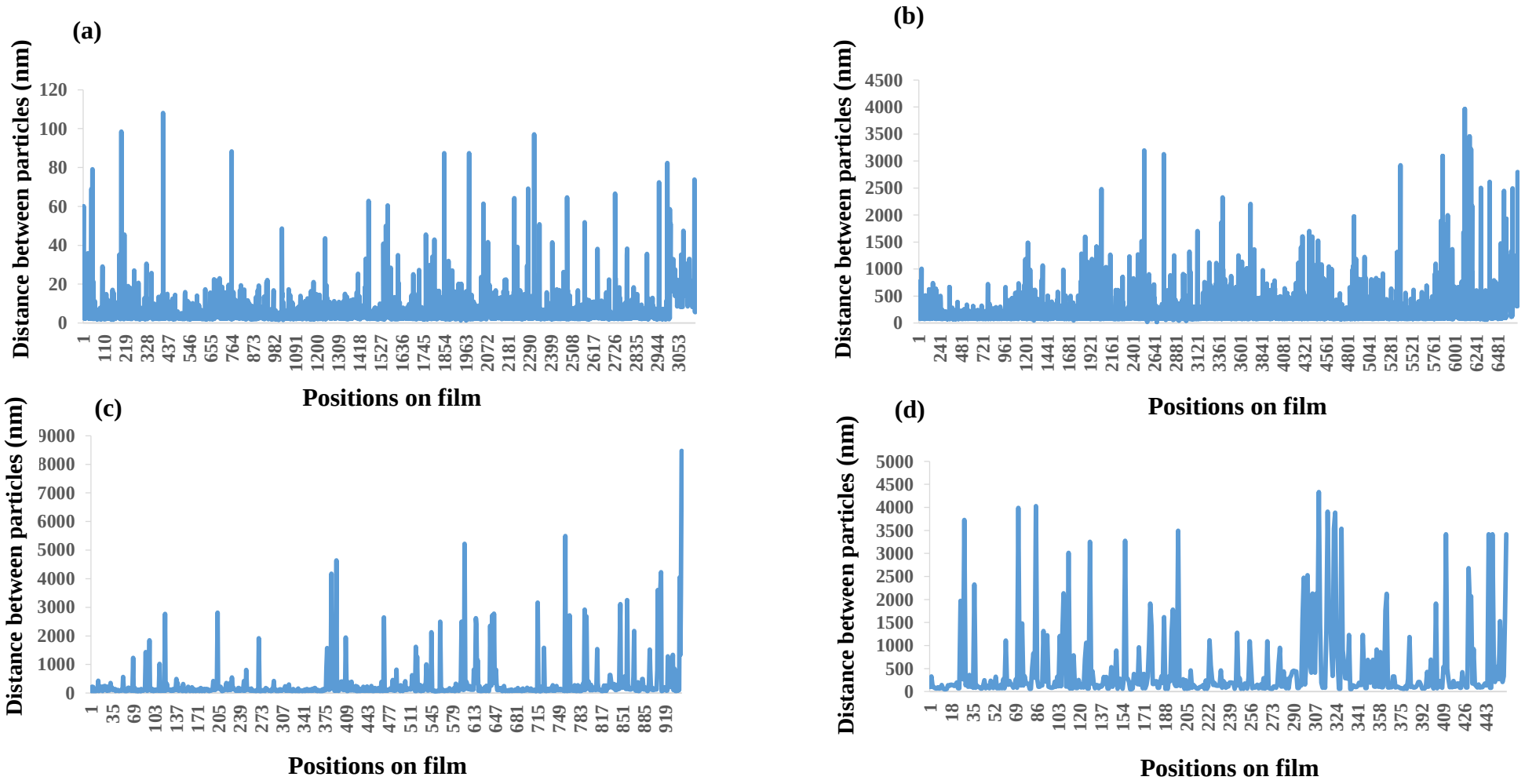
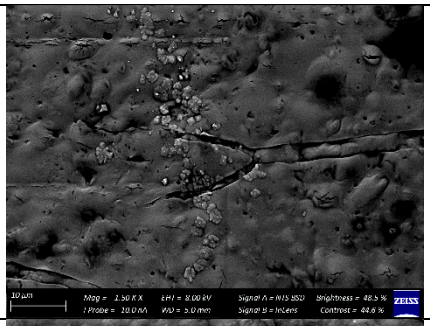
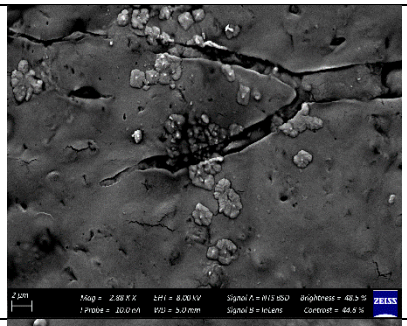
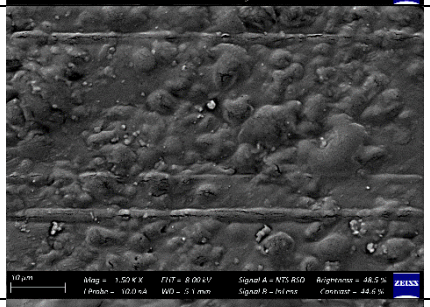
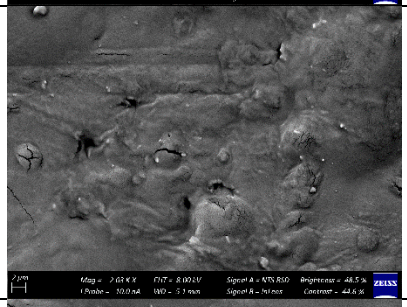
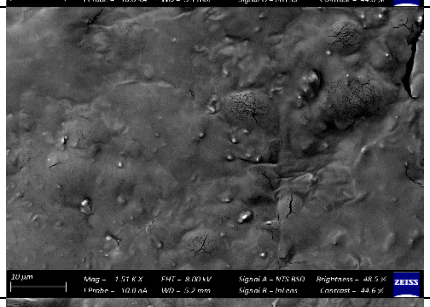
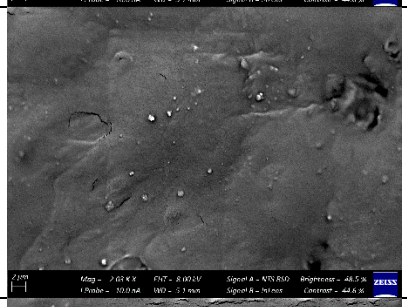
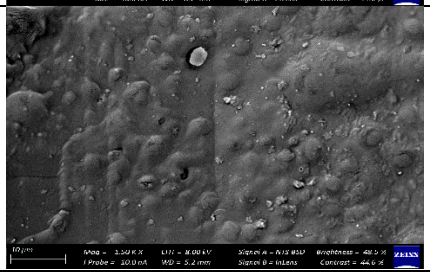
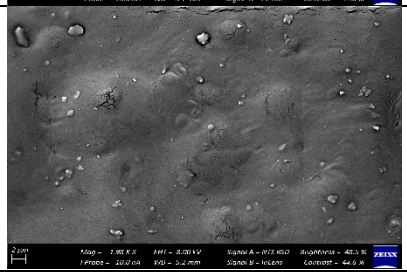


Fig. C1: Distances between nanocellulose particles in the films reinforced with nanocellulose with a degree of substitution of (a) 0, (b) 0.56 (c) 1.6 and (d) 2.34

Table C1: Dispersion of acetylated nanocellulose in acetylated hemicellulose films as affect by the degree of acetylation of nanocellulose

Reinforcing nanocellulose degree of acetylation	**Dispersion of nanocellulose in the film At different magnifications**		**Average distance between nanocellulose particles (nm)**
	x 1.50	x 2.00	
0			6.35
0.56			186.09
1.6			275.34
2.34			447.71

Appendix D: Polyphenol release and antioxidant activity of acetylated hemicellulose films and low density polyethylene films

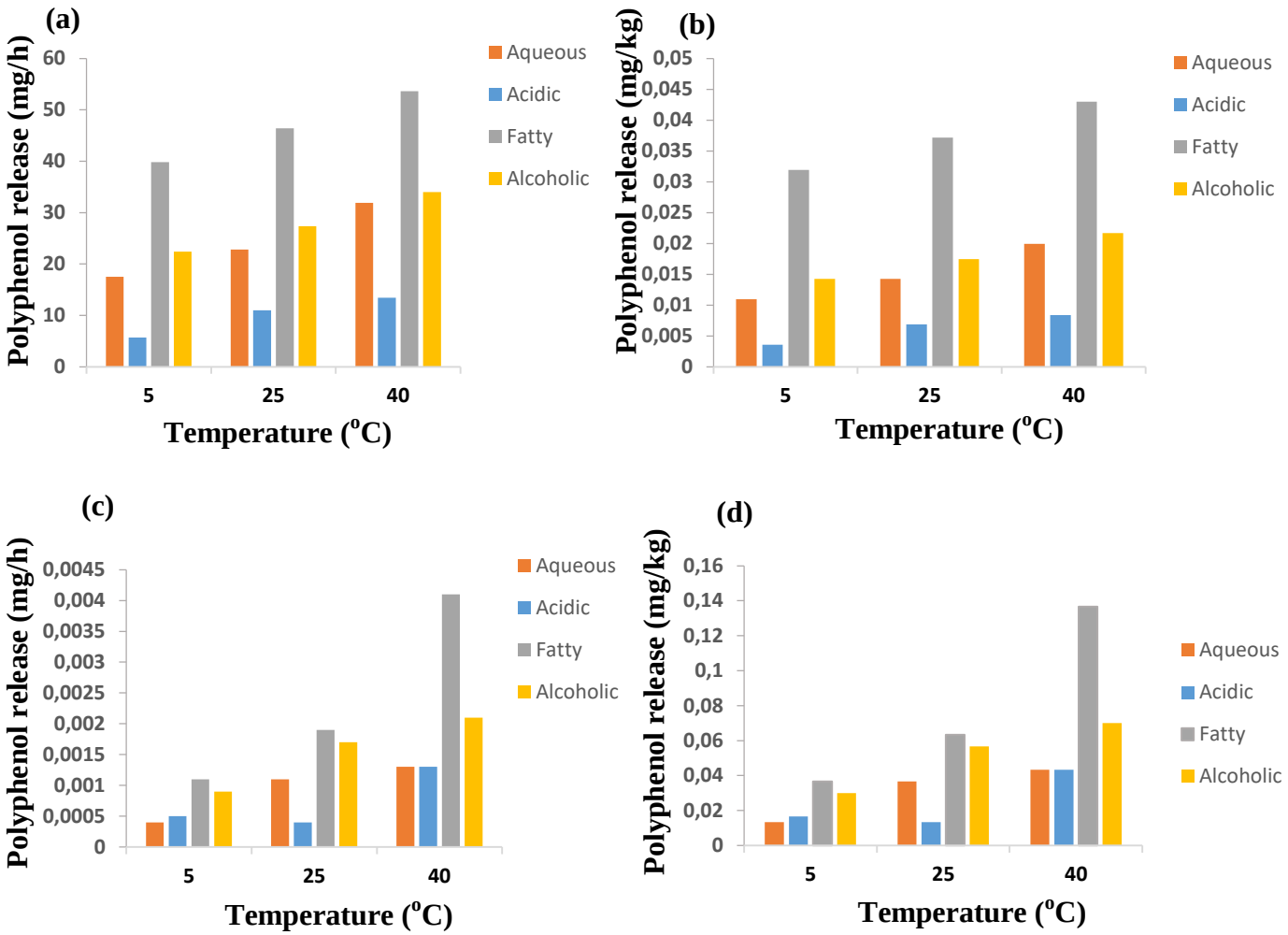


Fig. D1: Rate of polyphenol release by acetylated hemicellulose based films (a and b) and low density polyethylene films (c and d) into the food simulants in mg/h (a and d) and mg/kg of food simulant (b and d)

Table D1: Models and R^2 values for polyphenol release from hemicellulose based films into food simulants

Simulant type	Temperature (^oC)	Model	R^2 Value
Aqueous	5	$-0.0143x^2 + 1.0654x$	0.988
	25	$-0.0168x^2 + 1.2909x$	0.9414
	40	$4.7019\ln(x) + 9.5004$	0.9157
Acidic	5	$0.9411\ln(x) + 1.6265$	0.888
	25	$1.9508\ln(x) + 2.9869$	0.8588
	40	$2.2259\ln(x) + 7.2801$	0.8703
Alcoholic	5	$3.5118\ln(x) + 4.9143$	0.8376
	25	$4.6784\ln(x) + 8.1172$	0.9305
	40	$5.7584\ln(x) + 9.3018$	0.8376
Fatty	5	$6.6145\ln(x) + 13.15$	0.7575
	25	$8.2105\ln(x) + 14.745$	0.824
	40	$12.537\ln(x) + 29.915$	0.8399

Models were developed using Statistica version 7, 13.2 software

Table D2: Models and R^2 values for antioxidant activity of simulants in contact with hemicellulose films

Simulant type	Temperature (^oC)	Model	R^2 Value
Aqueous	5	$4.5245\ln(x) + 8.4753$	0.8948
	25	$3.9024\ln(x) + 9.1118$	0.9977
	40	$3.6579\ln(x) + 10.756$	0.9175
Acidic	5	$2.8865\ln(x) + 10.139$	0.8002
	25	$3.153\ln(x) + 11.498$	0.7401
	40	$3.9954\ln(x) + 11.79$	0.8563
Alcoholic	5	$4.6108\ln(x) + 10.292$	0.9937
	25	$6.5139\ln(x) + 12.613$	0.9373
	40	$0.0494\ln(x) + 3.3141x + 3.1054$	0.9624
Fatty	5	$2.4172\ln(x) + 6.6221$	0.9669
	25	$3.7186\ln(x) + 8.4847$	0.8424
	40	$6.6534\ln(x) + 9.2465$	0.6138

Models were developed using Statistica version 7, 13.2 software

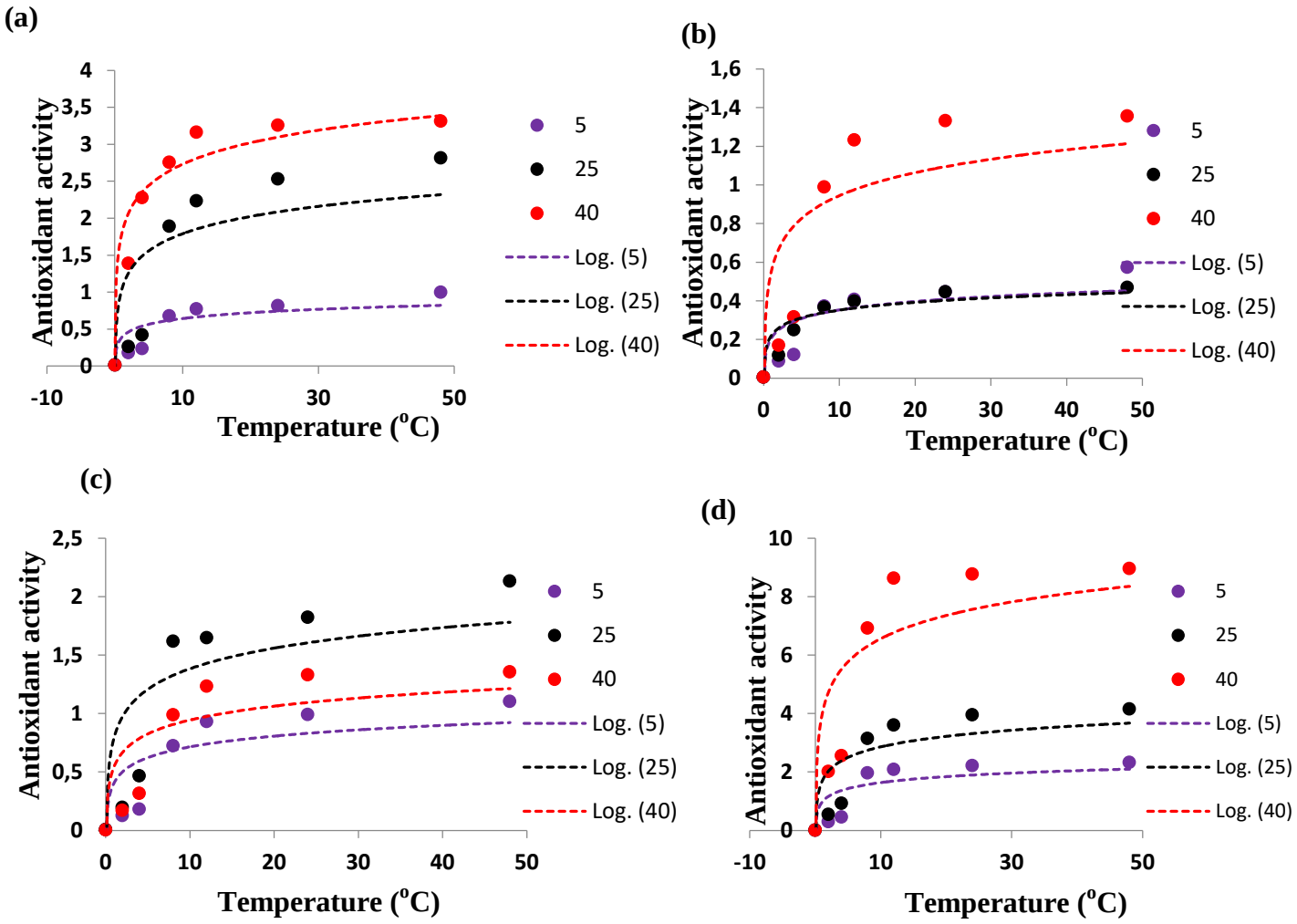


Fig. D2: Antioxidant activity of food simulants (a) aqueous (b) acidic (c) alcoholic and (d) fatty in contact with the low density polyethylene-based active packaging films

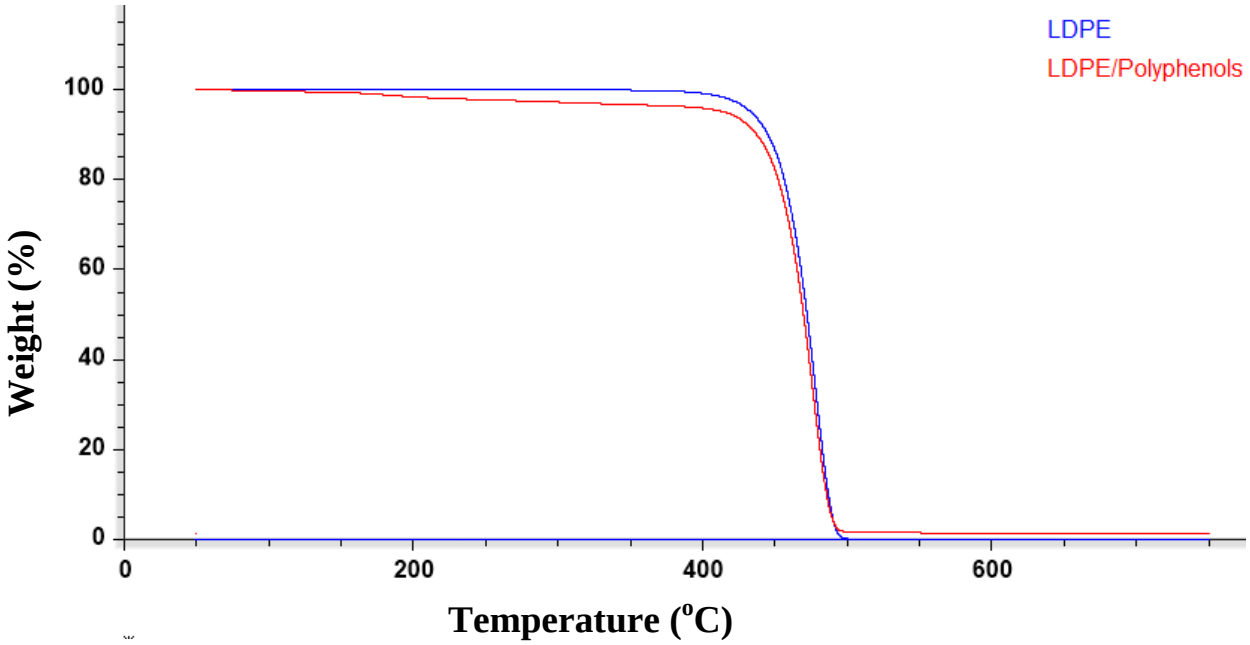


Fig. D3: Thermal stability of low density polyethylene films with and without mango peel polyphenols